RESPONSE OF ISOLATED LYSOSOMES TO VITAMIN A

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The nature of the barrier withholding lysosomal enzymes and the mechanisms involved in their release are thought to have significance for physiological and pathological processes (1). Dingle (2) and Fell *et al.* (3) have described the release of lysosomal enzymes in vitamin A–treated rat liver fractions. Because vitamin A results in rapid lysis or damage of erythrocytes (4, 5), nuclei, and mitochondria (6), it is believed to alter the structure of lipoprotein membranes. Although a lipoprotein membrane is believed to surround the lysosome, there is also evidence that lysosomes are complex glycolipoprotein granules in which acid hydrolases manifest latency because of ionic bonding (7, 8). To clarify the nature of the association of hydrolytic enzymes to the structure of the lysosome, an investigation was made of the simultaneous structural change by light-scattering and the release of free acid phosphatase activity that occurs in "mitochondria-free" suspensions of rat liver lysosomes upon treatment with vitamin A alcohol.

METHODS

Lysosomes were isolated from the livers of Sprague-Dawley albino male rats by the method of Sawant *et al.* (9) in 0.68 M sucrose + 1 mM EDTA. Acid phosphatase activity was assayed using *p*-nitrophenylphosphate as substrate. Incubations were made at 38°C for 10 minutes. The effects of alternate freezing and thawing, hypo-osmotic treatment, and incubation at 38°C on acid phosphatase of lysosomes were in agreement with those reported by Berthet *et al.* (10, 11).

Light-scattering changes at 546 mμ were measured at 90° with a Brice-Phoenix light-scattering photometer modified for recording as previously described (12). Additions of vitamin A alcohol, dissolved in 95 per cent ethanol, were made to lysosomal suspensions contained in the sample cuvette while

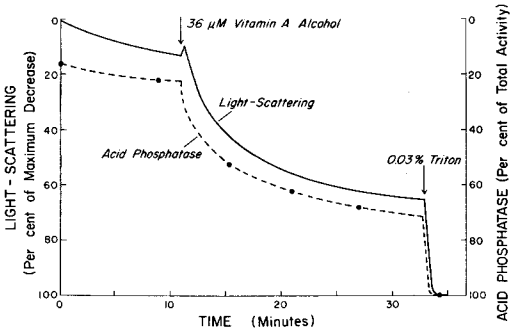

FIGURE 1 Effect of vitamin A alcohol on lysosomal light-scattering and free acid phosphatase activity. Lysosomes (2.4 mg protein) were suspended in 6.0 ml of 0.68 M sucrose + 1 mM EDTA at 35°C. Free acid phosphatase activity is expressed as per cent of total enzyme activity produced by the addition of Triton X-100. Initial light-scattering level taken as 100 per cent, and minimum level (16 per cent residual scattering) after Triton X-100 addition as 0 per cent.

light scattering was being continuously monitored at 35°C. For enzyme assays, sample aliquots were removed from the cuvette at various times and immediately added to incubation mixtures at 38°C. Control experiments were performed by adding equal volumes of 95 per cent ethanol.

RESULTS

The effect of vitamin A alcohol on lysosomal structure and acid phosphatase at 35°C is illustrated in Fig. 1. This experiment shows a typical recording of changes in light scattering and enzyme activity before and after the addition of 36 μM vitamin A alcohol to a lysosomal suspension. Dilution of the stock lysosomal suspension in reaction mixture is usually accompanied by a slow decline of light scattering (possibly indicating swelling) that is generally not associated with activation or release of acid phosphatase. This may be caused by the free lysosomal enzymes initially present in the system. An immediate acceleration of the slow light-scattering decrease follows vitamin A addition. A steady state is achieved in about 20 minutes, after the scattering level has declined by about 50 per cent. This large light-scattering decrease is almost exactly paralleled by increases in the per cent free acid phosphatase. Triton X-100 detergent (final concentration of 0.03 per cent v/v) was added to the reaction system in order to obtain a minimum light-scattering level (maximal structural change) and full release of acid phosphatase activity. Triton X-100 at this concentration has been shown by Wattiaux and de Duve (13) to produce maximum levels of free acid hydrolytic activity in lysosomal suspensions.

A closer examination of the effect of vitamin A alcohol on light-scattering levels and some control studies are shown in Fig. 2. The effect of vitamin A alcohol addition on lysosomal structure is not gradual but appears to become manifest after a critical concentration is reached. Activation of acid phosphatase also occurs only after the critical concentration is reached (not shown). Control additions of equivalent quantities of 95 per cent ethanol showed no change of light-scattering level or acid phosphatase. The minimum light-scattering levels and maximum enzyme activities produced by Triton treatment are identical to those obtained with lysosomal suspensions untreated with vitamin A alcohol. Also, light-scattering changes do not result from insolubility of vitamin A alcohol, since Triton X-100 addition still reduced light scattering to minimum levels. Keiser *et al.* (14) also did not observe turbidity changes with vitamin A concentrations less than 500 μM.

Control experiments were also performed to assess the degree of mitochondrial contamination of the lysosomal suspensions. Although a 6 to 10 per cent level of contamination by non-lysosomal particles has been reported for lysosomes prepared by this procedure (9), we were unable to obtain evidence for the presence of energy-dependent volume changes (with succinate, inorganic phos-

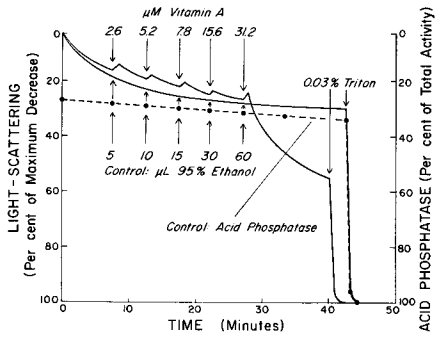

FIGURE 2 Titration of lysosomal light-scattering level with vitamin A alcohol. The reaction mixtures contained 2.4 mg lysosomal protein suspended in 6.0 ml of 0.68 M sucrose + 1 mM EDTA at 35°C. Acid phosphatase and light-scattering are expressed as in Fig. 1. The concentration of vitamin A alcohol added to the treated sample and the amount of 95 per cent ethanol added to the control are expressed cumulatively.

phate, and ATP) that are characteristic of mitochondria (12) or oxygen consumption in the presence of ascorbate plus tetramethyl phenylenediamine—an electron-feeder system that is a sensitive test for oxygen consumption by cytochrome oxidase in mitochondria or mitochondrial fragments (15). From the general effects of vitamin A alcohol on membranes, it would be expected that mitochondrial membranes, if present, would be disrupted by this substance. However, since mitochondria are not detectable, it may be concluded that the results obtained truly reflect modification of lysosomal membrane structure.

DISCUSSION

Conditions which release lysosomal hydrolases are associated with structural changes in lysosomes. Various treatments of lysosomes (including alternate freezing and thawing, incubation at 38°C, and addition of Triton X-100) which result in enzyme activation may also enhance enzyme activity by acting on the lipoprotein membrane, but the kinetics of structural changes accompanying activation of enzyme activity have not been clearly shown. Vitamin A alcohol is known to damage cell membranes and release lysosomal hydrolases *in vitro* and *in vivo* (4–6, 16–18). Also, Glauert *et al.* (5) reported in an electron microscope study of erythrocytes that the site of vitamin A action was at the membrane. Thus, the observation of the parallel nature of the decrease in lysosomal light scattering with release of acid phosphatase strengthens the proposal that lysosomes possess a membrane which contains the enzymes. The findings are not inconsistent with the proposal of Dingle *et al.* (5, 6, 19) that vitamin A acts by being incorporated into lipoprotein membranes. The observations reported here are also in agreement with those of Keiser *et al.* (14), who studied the effect of vitamin A on the enzyme activities and turbidity of lysosome-containing, large-granule fractions prepared from rabbit liver, and those of Weissmann *et al.* (20) who made similar studies with lysosomes from leucocytes. Hence, the light-scattering decreases observed in vitamin A–treated lysosomal suspensions support the concept (*cf.* Tappel *et al.*, 21) that swelling or membrane degradation is closely synchronized with enzyme activation.

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