THE ROLE OF PHOSPHOLIPIDS IN THE MANIFESTATION OF LYSOSOMAL ENZYME ACTIVITY

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UDC 612.014.015.12-06:/[612.397.7:547.953

Extraction of phospholipids, with 10% aqueous acetone, from liver lysosomes of rats leads to a sharp decrease (to 13.6% of the control) in activity of the membrane-bound lysosomal enzyme β-glucosidase but has virtually no effect on the activity of the soluble enzyme β-galactosidase or of acid phosphatase which is partially bound with the lysosomal membranes. Incubation of lysosomes with phospholipase C also induces a marked decrease in β-glucosidase activity (to 48% of the control after incubation for 5 min and to 30% after incubation for 120 min) and some decrease in acid phosphatase activity (to 70% of the control after 120 min), whereas β-galactosidase activity under these circumstances was virtually unchanged. Incubation of lipid-free (treated with aqueous acetone) lysosomes with nonpolar (Triton X-100, Tween-60) and polar (sodium cholate and sodium dodecylsulfate) detergents restores some of the β-glucosidase activity. The results indicate that phospholipids are essential for the manifestation of the catalytic activity of β-glucosidase.

Phospholipids (PL) play an important role in the function of membrane-bound enzymes of mitochondria [8, 9, 15] and the endoplasmic reticulum [7, 12, 14, 18]. However, the role of PL as regards lysosomal enzymes remains almost completely unstudied. Meanwhile, there is experimental evidence that some lysosomal enzymes are firmly bound with the membrane of these organelles [4, 7]. These membrane-bound enzymes include, in particular, β-glucosidase [7]. From recent investigations in the authors' laboratory, it seems that an essential condition for the manifestation of the catalytic activity of this enzyme is the formation of complexes between the enzyme protein of β-glucosidase and the components of the lysosomal membrane, primarily PL [5].

The object of this investigation was to study the possible role of PL in the manifestation of the catalytic activity of various lysosomal enzymes. Enzymes differing in the strength of their bond with the lysosomal membrane were chosen for investigation. These were β-glucosidase (3.2.1.21), an enzyme firmly bound with the lysosomal membrane [7], β-galactosidase (3.2.1.23), an enzyme similar to β-glucosidase functionally but, unlike the latter, an enzyme of the matrix [7], and acid phosphatase (3.1.3.2), an enzyme partly bound with the lysosomal membrane [17]. The chief techniques used were: a) a study of the activity of these enzymes in lysosomes from which the PL had been removed by extraction with acetone; b) a study of the activity of the same enzymes after incubation of the lysosomes with phospholipase C.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-300 g were used.

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Laboratory of Medical Enzymology, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. Department of Nutrition, Central Postgraduate Medical Institute, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 3, pp. 49-52, March, 1974. Original article submitted July 17, 1973.

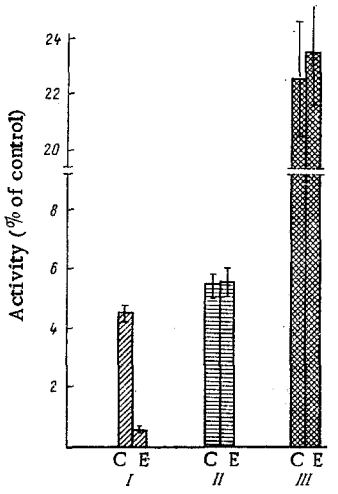

Fig. 1. Total enzyme activity in lipid-free lysosomes. C) control (intact lysosomes); E) experiment (lysosomes treated with 10% aqueous acetone). I) β-Glucosidase; II) β-galactosidase; III) acid phosphatase.

The lysosome fraction was isolated from the liver by the method of de Duve et al. [11] in 0.25 M sucrose with 0.001 M EDTA (pH 7.4). An enzyme control confirmed that the isolated fraction of lysosomes was sufficiently pure.

PL were removed from the lysosomes by mild extraction in the cold (0-2°C) with acetone containing 10% water by Fleisher's method [13] with certain modifications. To 27 ml of a mixture of acetone with water (155 : 11) was added 1 ml of a suspension of lysosomes (containing 20-30 mg protein) in cold 0.25 M sucrose. The mixture was stirred thoroughly and centrifuged for 10 min at 4000 g, the supernatant was carefully decanted, and the residue of lysosomes was resuspended in the original volume (1 ml) of 0.25 M sucrose. All extraction procedures did not take longer than 20 min.

To study the effect of phospholipase C on the behavior of the enzymes the suspension of lysosomes (30-40 mg protein/ml) was incubated at 37°C with a preparation of phospholipase C in the presence of 2.5 mM Ca^{++} and 0.08 M Tris-HCl buffer, pH 8.0. The phospholipase C (from Clostridium perfringens; Koch-Light, England) was added to the incubation medium to give a ratio of lysosomal protein : phospholipase protein of 20 : 1. At various times after the beginning of incubation (from 2 to 120 min), samples were taken from the incubation medium to test the activity of the lysosomal enzymes. The slowly cooled samples were treated with EDTA to a final concentration of 0.01 M to stop the action of phospholipase C.

Activity of β-glucosidase, β-galactosidase, and acid phosphatase and also the total protein content were determined by spectophotometric micromethods based on the use of biochemical ultramicroanalysis developed by Pokrovskii et al. [1-3].

EXPERIMENTAL RESULTS AND DISCUSSION

Data for the activity of the enzymes studied after extraction of PL from the lysosomes with acetone are given in Fig. 1. The β-galactosidase and acid phosphatase activity in lysosomes treated with aqueous acetone was virtually identical with their activity in the intact organelles. However, this treatment sharply reduced β-glucosidase activity, to 13.5% of the control.

The selective inhibition of β-glucosidase activity after treatment of the lysosomes with aqueous acetone was evidently due to removal of the PL from them. To confirm this hypothesis, another series of experiments was carried out to study the behavior of these enzymes after incubation of the lysosomes with phospholipase C, an enzyme removing the phosphorylated base from PL and thus sharply modifying their physicochemical properties. This approach is a more specific method of acting on the membrane PL than by their extraction [13].

Incubation of the lysosomes with phosphorylase C (Fig. 2) led to a marked decrease in β-glucosidase activity: by the fifth minute its activity was reduced to 48% of the control. Later during incubation β-glucosidase activity continued to fall (down to 30% of the control after incubation for 120 min), but the rate of decrease of its activity was much lower.

Incubation of the lysosomes with phosphorylase C also led to some decrease in the activity of acid phosphatase – an enzyme partly bound with the lysosomal membranes. However, toward the end of incubation acid phosphatase activity was reduced by only 30% compared with the control; moreover, the decrease in the activity of this enzyme, unlike that of β-glucosidase, took place more gradually and throughout the period of incubation. It is interesting to note that even after incubation of the lysosomes with phospholipase C for 120 min the activity of the soluble lysosomal enzyme β-galactosidase was not reduced.

These experiments thus showed that both the removal of PL from lysosomes by means of acetone and their destruction by phosphorylase C lead to a sharp decrease in the activity of β-glucosidase – an enzyme

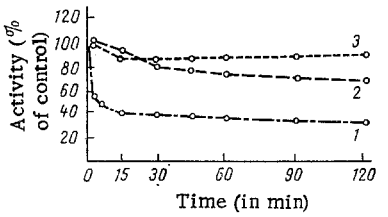

Fig. 2

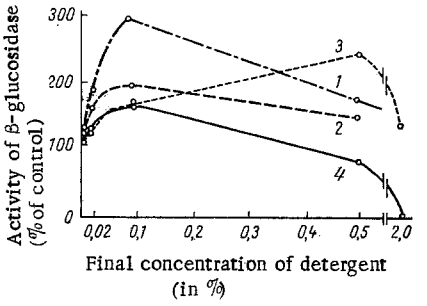

Fig. 3

Fig. 2. Effect of phospholipase C on lysosomal enzyme activity. Enzyme activity expressed in % of their activity in lysosomes not treated with phospholipase C.

Fig. 3. Activation of β-glucosidase of lipid-free lysosomes by detergents. β-Glucosidase activity expressed as a percentage of its activity in lipid-free lysosomes incubated under the same conditions with equivalent volumes of solvent (H_2O): 1) Triton X-100; 2) Tween-60; 3) sodium cholate; 4) sodium dodecylsulfate.

firmly bound with the lysosomal membranes. It can accordingly be concluded that PL of the lysosomal membranes are essential for the manifestation of the catalytic activity of that enzyme.

The PL are considered to be responsible for maintaining the catalytically active conformation of the enzymes of the mitochondria and endoplasmic reticulum produced by hydrophobic interactions between the enzyme proteins and PL [9, 16, 18]. Evidently a similar mechanism applies in the case of the lysosomal enzyme β-glucosidase.

Experiments were therefore carried out to study whether β-glucosidase in lipid-free lysosomes can be reactivated by means of detergents, compounds capable of active hydrophobic interaction with protein molecules (including enzyme proteins), coupled with changes in their conformation [10].

The action of two groups of detergents was studied: nonpolar (Triton X-100 and Tween-60) and anionic (sodium cholate and sodium dodecylsulfate). The lipid-free lysosomes were incubated (5 min, 0°C) with aqueous solutions of one of the detergents.

Incubation of lipid-free lysosomes both with nonpolar and with anionic detergents led to a marked increase in β-glucosidase activity. The strongest reactivating action was exhibited by Triton X-100: in a concentration of 0.1% of this detergent the β-glucosidase activity in the lipid-free lysosomes rose by 2.9 times compared with the control and reached 56% of its activity in the intact lysosomes. The reactivating effect of the other nonpolar detergent (Tween-60) was less marked (activation by 1.8 times in a concentration of 0.1%). The anionic detergents sodium cholate and sodium dodecylsulfate activated β-glucosidase by 2.3 and 1.8 times respectively.

It can be concluded from the results of these experiments that PL play an important role in the manifestation of the catalytic properties of the membrane-bound lysosomal enzyme β-glucosidase; their action is evidently aimed at maintaining the catalytically active conformation of this enzyme, and it is explained by hydrophobic interaction with the enzyme protein. At the same time, the fact that acid phosphatase activity is decreased by treatment of lysosomes with phospholipase C indicates that PL also play a role of some importance in the action of that enzyme. The results indicate that interaction of enzyme proteins with PL is evidently a characteristic feature of the membrane-bound enzymes of various cytological structures. Presumably interaction between the enzymes and PL constitutes one way by which the activity of the enzymes of subcellular structures can be regulated.

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