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STUDIES ON HUMAN POLYMORPHONUCLEAR LEUKOCYTE ENZYMES

III. DIFFERENTIAL ACTIVATION OF PRIMARY AND SPECIFIC GRANULES BY PHOSPHOLIPASE C AND DEOXYCHOLATE

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SUMMARY

1. Human polymorphonuclear leukocyte cytoplasmic extracts were preincubated at pH 7.2 in 0.34 M sucrose with exogenous phospholipase C or with deoxycholate, and immediately free and soluble activities were measured for several primary and specific granule enzymes.

2. Both treatments caused almost complete activation of latent lysosomal enzymes.

3. Specific granule membrane was strongly resistant to phospholipase C and deoxycholate treatments, because free and soluble alkaline phosphatase activity, used as marker enzyme for these granules, did not increase significantly.

4. The above results are evidence for a biochemical heterogeneity between primary and specific granule membranes.

INTRODUCTION

The structure-linked latency of lysosomal enzymes has been attributed to the existence around the lysosomes of a membrane-like barrier of lipoprotein nature restricting the accessibility of their internal hydrolases to external substrates [1].

Polymorphonuclear leukocytes contain two types of granules, primary and specific, which have separate origins and are different in nature. Primary granules represent a special type of primary lysosome containing peroxidase and lysosomal or digestive enzymes. They are produced early in development, from the concave face of the Golgi complex.

Specific granules are smaller, representing an entirely different secretory product which contains alkaline phosphatase and lysozyme [2] and collagenase [3], lacking lysosomal enzymes and peroxidase. They are produced later in development during the myelocyte stage, and arise from the opposite or convex face of the Golgi complex [4, 5].

On the other hand, it has been recently reported in rabbit polymorphonuclear leukocytes that although primary and specific granule membrane were quite similar

in ultrastructural appearance, they showed distinct differences in cholesterolphospholipid ratios and in protein components [6], suggesting a biochemical heterogeneity between the two types of membranes.

In this paper we studied the effects of exogenously added phospholipase C (EC 3.1.4.3) and deoxycholate on the free activities of primary and specific granule enzymes and present evidence for a biochemical heterogeneity in the properties of primary and specific granule membranes.

MATERIALS AND METHODS

Chemicals

Cacodylic acid (sodium salt), deoxycholic acid (sodium salt) and phospholipase C (Type I) were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Substrates

p-Nitrophenyl-α-D-mannoside, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide and phenolphthalein glucuronic acid (sodium salt) were from Sigma Chemical Co. and β-glycerophosphate (disodium salt) from Merck, Darmstadt, Germany.

Preparation of polymorphonuclear suspensions

Leukocytes were isolated from the blood of fasting normal subjects and processed as previously described [7]. About 92–95% of the cells in the final suspension were polymorphonuclear leukocytes.

Enzyme assays

All enzymes were assayed according to the techniques described by Avila and Convit [7, 8]. Free activities were measured in the presence of 0.34 M sucrose at 37 °C for 1 h.

Preincubations with phospholipase C or deoxycholate

All these experiments were performed on cytoplasmic extracts prepared in ice-cold 0.34 M sucrose–50 mM KCl as reported previously [9], except that heparin was omitted because the sonication procedure was sufficient to obtain total cell rupture.

For the phospholipase C experiments, 40 mg of a fresh cytoplasmic extract were preincubated for 30 min at 25 °C in a total volume of 1 ml containing 0.34 M sucrose, 15 mM cacodylate–HCl buffer (pH 7.2) and 4 mM $CaCl_2$ to activate the enzyme. Phospholipase C activity was stopped by adding 0.2 ml of cold 100 mM EDTA (pH 7.2) in 0.34 M sucrose, and by immediately diluting the preincubation mixture 8 times with cold 0.34 M sucrose. Control tubes were treated similarly, except that 0.34 M sucrose was added to the preincubation mixture instead of phospholipase C.

In other experiments, 50 mg of a total homogenate was treated with phospholipase C as for free activities, and a supernatant fraction was isolated by centrifuging at 59 000 × *g* 10 min at 4 °C in a Beckman L2-65B centrifuge. The deoxycholate experiments were carried out exactly as described in the phospholipase C procedure, except that preincubation was carried out at 4 °C and no $CaCl_2$ or EDTA were added.

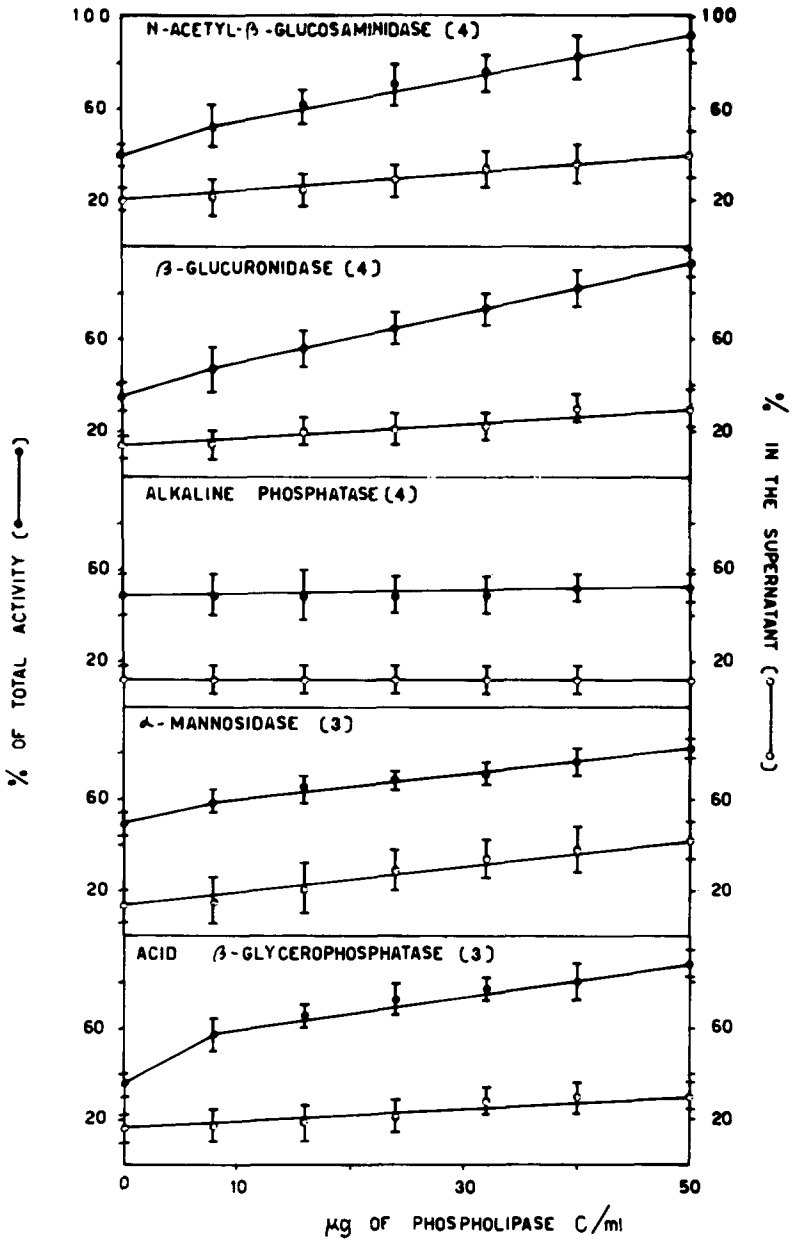

Fig. 1. Activation of human polymorphonuclear leukocyte hydrolases by increasing concentrations of phospholipase C. For details see text. All results are expressed in percentage of the activities measured in the presence of 0.04% (v/v) Triton X-100. Numbers in parentheses refer to number of experiments.

Total activities were measured in cytoplasmic extracts treated exactly as for free activities, but in the presence of 0.04% Triton X-100.

RESULTS

Effect of phospholipase C treatment

Preincubation of a human polymorphonuclear leukocyte cytoplasmic extract in 0.34 M sucrose at pH 7.2 and in the presence of phospholipase C (0–50 μg/ml) caused practically complete activation of the several latent primary granule enzymes measured. Similar results have been published for rat liver and brain lysosomes [10, 11]. In the case of alkaline phosphatase activity, used to determine specific granule latency, no activation was found in the concentration range used.

In these experiments the free activities of the control tubes were always higher than those recently reported [9], this fact is due perhaps to the preincubation procedures to which the cytoplasmic extracts were submitted.

The activation of primary granule enzymes was found to be associated with a partial liberation of the enzymes into the solution. Thus, acid hydrolase-soluble activities slowly increased in a parallel fashion to the free activities but they did not reach values higher than 45% of the homogenate total activity. Again, no increase was found in the percentage of soluble alkaline phosphatase (Fig. 1).

The total activities of the several hydrolases studied suffered no change under the influence of the phospholipase C treatment.

Effect of deoxycholate treatment

Preincubation of a human polymorphonuclear leukocyte cytoplasmic extract in 0.34 M sucrose at pH 7.2 and 4 °C in the presence of deoxycholate 0–0.02% caused almost complete activation of the several lysosomal enzymes measured.

Again no increase in the alkaline phosphatase free activities was found in the concentration range used (Fig. 2).

The total activities of the several hydrolases studied suffered no significant change under the influence of the deoxycholate treatment.

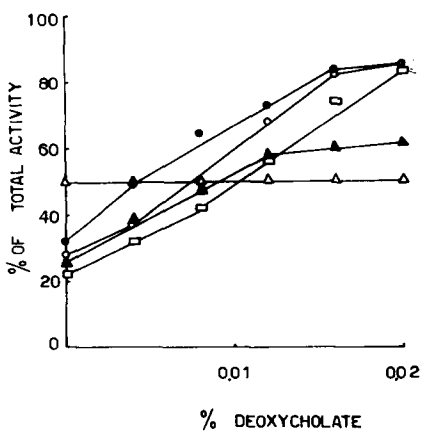

Fig. 2. Influence of deoxycholate concentration on release of human polymorphonuclear leukocyte hydrolases. ○–○, β-glucuronidase; ●–●, α-mannosidase; △–△, alkaline phosphatase; ▲–▲, acid β-glycerophosphatase; □–□, *N*-acetyl-β-glucosaminidase.

DISCUSSION

Data reported in this study indicate that pretreatment of cytoplasmic extracts with phospholipase C or deoxycholate caused a gradual access to the substrates of the several lysosomal hydrolases studied, but not of the alkaline phosphatase.

The activation curves of the four lysosomal enzymes showed a fairly close parallelism and differed significantly in both experimental conditions from the corresponding curve obtained for the specific granule alkaline phosphatase.

Beaufay and deDuve [10] have already discussed the methodology used in phospholipase C experiments, and suggested that it is vitiated to some extent by artifacts which tend to exaggerate the differences between free activities and solubilization. Owing to adsorption phenomena, the values for physical release (solubilization) are underestimated; on the other hand, the accessibility of the enzymes (free activities) may be over estimated if further disruption of the particles occurs during the assay themselves.

The possibility of partial adsorption of the acid hydrolases released from primary granules in the phospholipase C experiments was studied by washing the sedimented granular fraction several times with the homogenization medium. This procedure, however, did not increase the percentage of the acid hydrolases present in the supernatant, suggesting that it is not probably the cause of the differences between the free activities and solubilization, and consequently giving indirect evidence that the accessibility of the enzymes may be overestimated.

Acid hydrolases reacted almost fully in the free activity assays, either because their release is completed under the combined effects of the acidity and temperature prevailing during these assays, or because their substrates are able to penetrate freely within the particles in which they are trapped. Whichever the reason, it is obvious that all the acid hydrolase-containing particles have been injured by the phospholipase C treatment.

That specific granules were not activated by phospholipase C treatment could be readily interpreted as a consequence of a strong resistance to an enzymatic disorganization of the lipoprotein barrier of the specific granules. This fact and the strong resistance to the deoxycholate treatment also observed could be attributed either to the degree of accessibility of membrane phospholipids to the externally added activating substances, to a characteristic biochemical composition of the specific granule membrane or to a combination of both possibilities.

Regarding the second possibility, it has been recently reported in rabbit polymorphonuclear leukocytes that the cholesterol to phospholipid molar ratios of the primary and specific granules membrane fraction are different [6].

Finally, we believe this paper adds more evidence indicating either that the Golgi apparatus consists of heterogeneous membrane; that, in particular, considerable differences in membrane composition may exist between the inner cisternae where the primary granules are formed, and the outer ones from which the specific granules originate; or that the molecular structure of the entire Golgi apparatus undergoes considerable change when promyelocytes mature into myelocytes [6].

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REFERENCES

1 De Duve, C. (1963) in Lysosomes (De Reuck, A. V. S. and Cameron, M. P., eds) p. 1, Ciba Found Symp., Churchill, London
2 Baggiolini, M., Hirsch, J. G. and De Duve, C. (1969) J. Cell Biol. 40, 529–540
3 Robertson, P. B., Ryel, R. B., Taylor, R. E., Shyu, K. W. and Fullmer, H. M. (1972) Science 177, 64–65
4 Bainton, D. F. and Farquhar, M. G. (1966) J. Cell Biol. 28, 277–293
5 Bainton, D. F., Ullyot, J. L. and Farquhar, M. G. (1971) J. Exp. Med. 134, 907–934
6 Nachman, R., Hirsch, J. G. and Baggiolini, M. (1972) J. Cell Biol. 54, 133–140
7 Avila, J. L. and Convit, J. (1973) Biochim. Biophys. Acta 293, 397–408
8 Avila, J. L. and Convit, J. (1973) Clin. Chim. Acta 47, 335–345
9 Avila, J. L. and Convit, J. (1973) Biochim. Biophys. Acta 293, 409–423
10 Beaufay, H. and de Duve, C. (1959) Biochem. J. 73, 604–609
11 Koenig, H. and Gray, R. (1964) J. Cell Biol. 23, 50A