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Subcellular localization of acid hydrolases in rat lungs

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SUMMARY

The subcellular distribution of twelve acid hydrolases was studied in rat lung homogenates. The distribution profile of all the hydrolases, except cathepsin C (EC 3.4.4.9), is simlar to that of lysosomal enzymes in rat and guinea pig liver. The microsomal marker, glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9), and mitochondrial marker, the succinate oxidase system, were localized mainly in their respective fractions. The increases of specific activities of the light-mitochondrial fraction over the homogenate specific activities are similar to those obtained for rat liver and indicates the usefulness of the differential centrifugation method described for preparation of acid hydrolase-rich fractions from lung homogenates. This study also shows some qualitative similarities in the hydrolase complements of lung and liver.

Studies on the pathogenesis of certain pulmonary diseases and the effects of oxidant pollutants on the biochemistry of the lungs have stimulated interest in the lysosomal hydrolases of lungs[1]. Lysosomes, which contain a full complement of hydrolases, could play a critical role in the response of the cells to ingested particulate matter and to damage brought about by oxidant pollutants. Most studies on lysosomes have been carried out on liver[2–4], kidney[5], spleen[6] and brain[7], and information concerning the fractionation and the enzymatic complement of lysosomes in lungs is limited. This study provides information on the preparation of a fraction that contains lysosomes from rat lung homogenates and its complement of hydrolases.

Lungs that were removed immediately from phenobarbital-anesthetized, 1-month-old Sprague–Dawley rats were freed of connective tissue, minced and homogenized in 5 vol. of 0.3 M sucrose and 1 mM EDTA, pH 7.0, using a motor driven Teflon pestle in a

Potter–Elvehjem homogenizer. The subcellular fractions were obtained by a modification of the procedure of de Duve *et al.*[2] for rat liver. Centrifugation was done in the following manner: nuclear and cell debris, 600 x *g* for 10 min; heavy mitochondria, 3500 x *g* for 10 min; light mitochondria, 57 000 x *g* for 7 min; microsomal pellet, 110 000 x *g* for 60 min; the supernatant was collected as the soluble fraction.

Lysozyme[8] (mucopeptide *N*-acetylmuramylhydrolase, EC 3.2.1.17), cathepsin A[9], glucose-6-phosphatase[10] (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) and the succinate oxidase system[11] were assayed according to published procedures. Acetylphenylalanyltyrosine hydrolase was assayed by a variation of a method described by Menzies and McQuillan[12]. The determinations of the other hydrolases are described elsewhere[13,14].

The distribution of the activities among the subcellular fractions of the hydrolases (lysosome markers), glucose-6-phosphatase (microsomal marker) and the succinate oxidase system (mitochondrial marker) is shown in Fig. 1. Each hydrolase, except cathepsin C

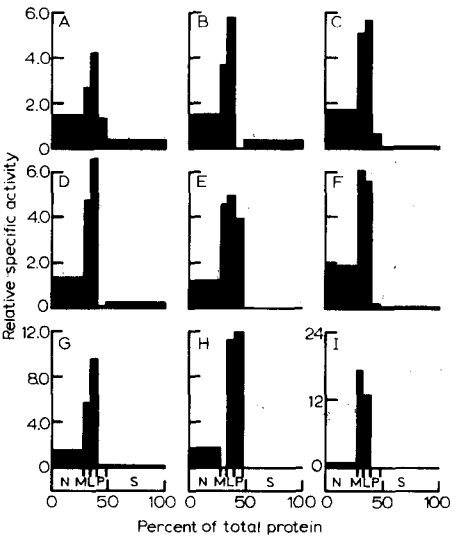

Fig. 1. Subcellular distribution of (A), acid phosphatase, (B) cathepsin B2, (C) β-*N*-acetylglucosaminidase, (D) cathepsin A, (E) *N*-acetylphenylalanyltyrosine hydrolase, (F) cathepsin C, (G) cathepsin D, (H) glucose-6-phosphatase and (I) succinate oxidase system. The fractions are: N, nuclear; M, heavy mitochondrial; L, light mitochondrial–lysosomal; P, microsomal; S, soluble. The relative specific activity is defined as % total activity/% total protein.

(EC 3.4.4.9), exhibited the highest relative specific activity in the light-mitochondrial fraction. The distribution profile is similar to that of lysosomal hydrolases in rat liver[2] and guinea pig liver[15]. The combined activities of the light- and heavy-mitochondrial fractions accounted for 25 to 60% of the total activities of the hydrolases. The recoveries of the enzyme activities varied from 46 to 86%, while better than 95% of the protein was recovered. Glucose-6-phosphatase and succinate dehydrogenase were found localized mainly in the microsomal and heavy-mitochondrial fractions, respectively.

The specific activities in the fractions containing lysosomes and the homogenates are shown in Table I. The enrichment of the lysosomal activity over the homogenate varied from 3.6-fold for β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) to 15.7-fold for cathepsin D (EC 3.4.4.23). Increases of 4- to 14-fold have been obtained for hydrolases in fractions containing lysosomes prepared by differential centrifugation from rat liver.

Since no attempt was made in this study to separate the different cell types in lungs, the source of the hydrolase activity represents a mixed population[16]. Cytochemical

TABLE I

SPECIFIC ACTIVITIES OF ACID HYDROLASES IN RAT LUNG

The values are averages ± standard deviations of three replicates done on a sample pooled from 14 rat lungs.

Enzyme (substrate)	*Specific activity (nmoles product released/min per mg protein)*		*Increase over homogenate (-fold)*
	Homogenate	*Light-mitochondrial and lysosomal fraction*	
Acid phosphatase (β-glycerophosphate)	18.3 ± 2.4	88.3 ± 5.8	4.8
Cathepsin C (glycyl-L-tyrosine amide)	40.2 ± 6.0	174.8 ± 6.6	4.4
Cathepsin D (denatured hemoglobin)	4.8 ± 1.2	75.6 ± 11.0	15.7
Cathepsin B2 (benzoylarginine amide)	4.9 ± 0.4	38.0 ± 4.0	7.8
Cathepsin A (carbobenzoxy-α-L-glutamyl-α-L-phenylalanine)	7.4 ± 1.6	63.6 ± 2.9	8.6
Acetylphenylalanyltyrosine hydrolase (*N*-acetyl-α-L-phenylalanine–α-L-tyrosine)	1.8 ± 0.8	12.4 ± 5.2	6.7
β-*N*-Acetylglucosaminidase★ (*p*-nitrophenyl *N*-acetyl-β-D-glucosaminide)	15.8 ± 0.6	125.5 ± 4.8	7.8
Lysozyme (*M. lysodeikticus*)★★	156 ± 1.0	759 ± 21.5	4.9
β-Galactosidase★ (*p*-nitrophenyl β-D-galactoside)	1.0 ± 0.08	5.2 ± 0	5.2
β-Glucosidase★ (*p*-nitrophenyl β-D-glucoside)	0.26 ± 0.02	0.95 ± 0.05	3.7
Arylsulfatase★ (*p*-nitrocatechol sulfate)	2.0 ± 0.03	14.7 ± 0.6	7.3
β-Glucuronidase (*p*-nitrophenyl-β-D-glucuronide)	2.7 ± 0.2	9.8 ± 0.1	3.6

★β-*N*-Acetylglucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30).
β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23).
β-Glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21).
Arylsulfatase (aryl-sulfate sulfohydrolase, EC 3.1.6.1).
★★Units/mg protein; 1 unit = 0.001 absorbance change/min.

evidence shows a high amount of acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) in the alveolar macrophages and less in the Type II epithelial cell[16–18]. The peculiar distribution of cathepsin C may indicate that its lysosomal source belongs to a cell type different from that of the other hydrolases.

The typically lysosomal distribution of the hydrolases and the several-fold increase of the activities over the homogenate indicate the suitability of using the differential centrifugation employed in this study to prepare subcellular fractions from rat lungs rich in acid hydrolases. This study also reveals the qualitative similarities in the hydrolase complement of rat lung with liver, although they may differ in quantities.

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