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Note

Separation of rat thyroid lysosome subpopulations on Sepharose 2B

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Although gel chromatography is widely used for molecular fractionation, it has rarely been used for subcellular fractionation. This paper describes an attempt to separate a crude thyroid lysosomal population into subpopulations, characterized by the criteria of age and size, using Sepharose 2B gel.

Thyroid lysosomes are involved in the degradation of thyroglobulin (Tg), an iodinated glycoprotein that is the source of thyroid hormones. The intralysosomal hydrolysis of Tg is known to be heterogeneous, as recently iodinated (new) Tg is hydrolysed faster than the previously iodinated (old) Tg[1,2]; thus, one can consider that, taking the iodine specific radioactivity (SRA = new label/old label) as an index, the higher the SRA the younger is the lysosome. The size distribution of organelles was studied by morphometry. A relationship between the size distribution of the lysosomes and the mean "age" of their iodine content has been found and could be interpreted as separation of the lysosomes into physiological subpopulations.

MATERIALS AND METHODS

Biological material

Male Wistar rats, weighing about 250–300 g and housed at 23 ± 1°, received 50 μg of iodine daily. They were maintained in isotopic equilibrium with ^{125}I as described by Simon[3]. Two hours before killing they received one intraperitoneal injection of 250 μCi of carrier-free ^{131}I in 0.5 ml of 0.9% sodium chloride solution. Thyroid lobes were homogenized in a Tris·HCl (0.1 *M*)–saccharose (0.15 *M*) medium of pH 7.0, and the bulk of iodinated particles was prepared as described by Simon *et al.*[4]. Briefly, a 34,000 *g* lysosomal pellet was obtained by re-suspending the washed pellet in the above elution medium.

Gel chromatography

Sixty grams of Sepharose 2B (Pharmacia, Uppsala, Sweden) were washed three times by gentle agitation with distilled water in order to discard the azide. After re-suspension in the elution medium, the gel was poured into a glass column of I.D. 2.5 cm. All of the experiments were performed at 4°.

In some experiments, the void volume (V_0) of the column was determined before chromatography by measuring the elution volume of a 2% solution of Blue Dextran 2000 (Pharmacia).

The total population of lysosomes (2-ml sample) of at least four pooled thyroids was applied on to the column and eluted with about 100 ml of the elution medium. Fractions of 20 drops were collected with a Gilson fraction collector. The complete organic iodine recovery was obtained only after a second elution with the elution medium containing 1% Triton X-100.

Measurement of radioactivity

The ^{131}I and ^{125}I activities of each fraction collected were measured using a two-channel Packard Autogamma spectrometer. The ^{127}I content of each fraction was calculated from its ^{125}I activity as described by Simon[3], and the distribution of old (^{127}I) and new (^{131}I) iodine was followed with respect to their specific radioactivity (SRA = parts per 1000 of ^{131}I injected per microgram of ^{127}I).

Morphometric study

Aliquots of the first subpopulation of lysosomes eluted in the void volume of the column (L_I) and the total population (L_T) were centrifuged for 1 h at 80,000 *g*. Each pellet was immersed at 4° for 1 h in 2 ml of 2% gluteraldehyde solution in 0.1 *M* phosphate buffer (pH 3), post-fixed in 1% osmium tetroxide solution and embedded in Araldite. Sections 300–600 Å thick were prepared with a LKB microtome and stained with 2% potassium permanganate solution.

The morphology of the pellet was observed with a Hitachi HU 12 electron microscope. The morphometric study was carried out on photographs taken at the final magnification of 33,000 from each field selected at random. Each photograph was superimposed at random with a network of parallel lines, and the number and the length of intersections between these lines and organelle sections were recorded. For each population, results from about 1000 measurements were totalled and used for the calculation of its representative distribution of organelle diameters. The method of calculation will be published elsewhere[5].

RESULTS

The lower part of Fig. 1 shows the results of thyroid lysosome chromatography on a sample from four rats in isotopic equilibrium. The ^{125}I label permits the determination the iodine pool within each particulate subpopulation: (1) the first subpopulation (L_I) was eluted in the void volume (V_0) and represents $17.67 \pm 4.43\%$ ($n = 9$); (2) the second subpopulation (L_{II}) was eluted at about $2V_0$ and represents $15.17 \pm 13.54\%$ ($n = 5$); (3) the third subpopulation (L_{III}) is strongly bound to the gel, its iodinated content being discharged only after solubilization of lysosomal membranes with Triton X-100, and represents $46.31 \pm 2.72\%$ ($n = 5$). The fractions between the peaks represent about 20% and complete recovery of iodine ($99.0 \pm 1.1\%$) ($n = 5$) is therefore obtained.

The recovery of L_I is independent of the sample size as the mean recovery is always about 18% ($18.21 \pm 2.43\%$) ($n = 5$) for samples obtained from 4, 7, 28 and 36 rats.

A comparison of the iodine SRA of each subpopulation, as shown in the upper part of Fig. 1, demonstrates that SRA (L_I) > SRA (L_{II}) > SRA (L_{III}). This result is obtained after a single injection of ^{131}I 2 h before killing.

Fig. 2 shows the frequency of *true* organelle diameters calculated from the

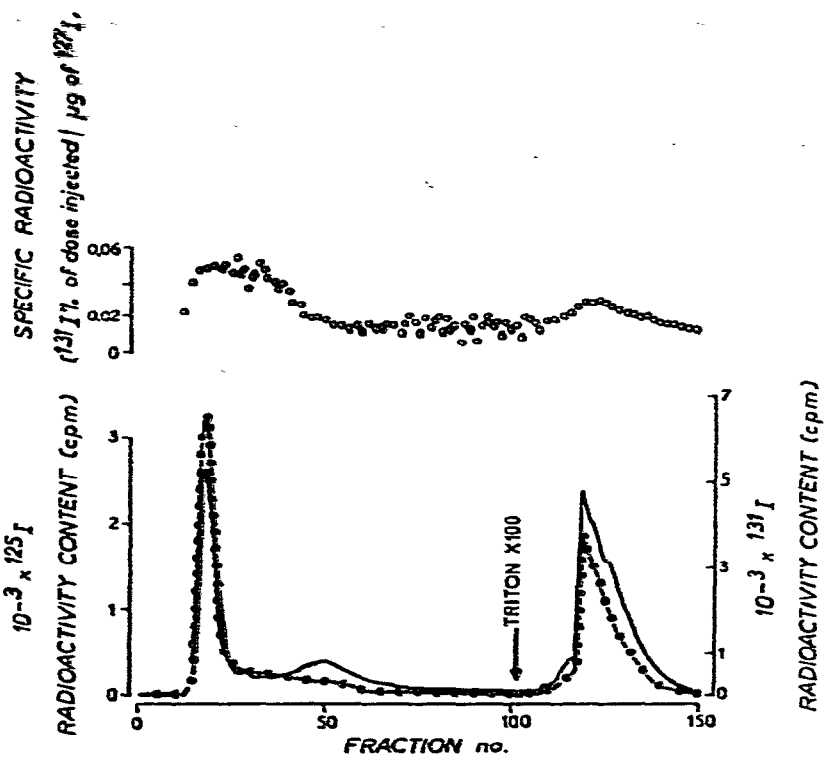

Fig. 1. Fractionation of a total population of thyroid lysosomes with Sepharose 2B. Four rats, isotopically equilibrated with ^{125}I, were injected with 250 μCi of ^{131}I 2 h before killing. The total population of lysosomes was layered on the chromatographic column equilibrated with 0.15 *M* sucrose buffered with 0.1 *M* Tris·HCl (pH 7). Elution was carried out successively with the same medium (100 ml) and the same medium containing 1% of Triton X-100 (500 ml). In the lower diagram, the ^{125}I (———) and ^{131}I (●—●) profiles show the successive elution of three subpopulations of lysosomes, L_I, L_{III} and L_{II}. The ^{125}I content of each fraction was calculated, then its specific radioactivity (SRA) was calculated with respect to ^{131}I. The upper diagram shows that the L_I subpopulation contains the higher proportion of newly iodinated molecules.

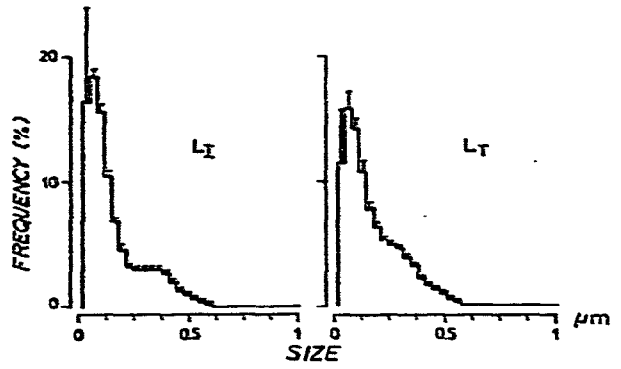

Fig. 2. Morphometry of the total population and subpopulation L_I of rat thyroid lysosomes. Each histogram gives the frequency of each size class of organelles calculated from observed sizes. The bars indicate standard errors for the classes. The ranges of *true* diameters are similar but the profiles are different.

observed diameters of the total population L_T and the subpopulation L_I. Both populations have a true diameter in the range 0.03–0.6 μm range. The maximum frequency for both L_T and L_I is observed at an organelle diameter between 0.03 and 0.07 μm. but there are 20% less organelles in the range 0.25–0.40 μm for L_I compared with L_T. This is statistically significant at the level $P < 0.05$ (t-test).

DISCUSSION

A crude thyroid lysomal population can be separated into three main subpopulations by Sepharose 2B chromatography. The delayed elution of L_{III} and the adsorption of L_{II} could be a function of the size of the organelles. Systematic studies by Gerlich *et al.*[6] of the elution of particles of known sizes on Sepharose 2B have shown that (1) K_{av} diminishes as their size increases and (2) only organelles of maximum diameter 0.08 μm were eluted with total recovery, and those of diameter over 0.08 μm were adsorbed to the gel; 80% of 1-μm diameter organelles were eluted compared with only 50% of 0.3-μm organelles. Their results indicate that only the smallest organelles (maximum diameter 0.1 μm) are entirely represented in L_I and that L_{III} consists mainly of the largest organelles. Our results also suggest that L_{II} is composed of vesicles with diameters in the range 0.2–0.5 μm. Hence Sepharose 2B chromatography would roughly separate lysosomes into three subpolulations with $L_I < L_{II} < L_{III}$ intems of their size. This is confirmed, at least in part, by our morphometric study in which we have pointed out that if L_I is composed of all organelle sizes it is significantly low in 0.25–0.40-μm organelles.

Moreover, the proposed method permits the separation of lysosome subpopulations according to their "age". Thus, taking the SRA as an index, the youngest lysosomes are the smallest and the oldest are the largest, as SRA (L_I) > SRA (L_{II}) > SRA (L_{III}). As we have previously suggested[7], it can be proposed that successive fusion of lysosomes between themselves leads to their progressive maturation, as revealed by the size increase that is associated with a decrease in content of young ioninated molecules. The biological implication of this interpretation will be published elsewhere.

In conclusion, Sepharose 2B chromatography is a useful method for the fractionation of the thyroid lysosomal subpopulations. It is interesting that, for a given gel volume, column size and sample volume, and with the concentration of the sample loaded on to the column varying by a factor of 9, the recovery of lysosomal iodine was complete and the recovery of each subpopulation was neither drastically nor systematically modified. Therefore, this method is highly reproducible and the origin of the fluctuation seems to be biological rather than technical. Moreover, although at least one subpopulation (L_{II}) can never be recovered as integral particles, it should be emphasized that the use of a pure lysosomal sample will permit comparative studies between thyroid lysosomal subpopulation contents: soluble molecules (iodinated molecules, enzymes) or solubilized molecules derived from membranes.

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