Isopycnic Equilibrium of Mouse Liver [^{131}I]Albumin Digesting Particles in Sucrose Gradients *

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Z. Naturforsch. **33 c**, 216–218 (1978) ; received December 30, 1977

Lysosomes, Digestive Vacuoles, Intracellular Hydrolysis

The isopycnic equilibrium of [^{131}J]albumin digesting particles from mouse liver homogenates occurs, in a sucrose gradient, at a density 1.25, and it does not change with time after albumin injection (5 to 300 minutes).

Intravenously injected [^{131}I]albumin (RISA) is digested in subcellular particles of mouse [1, 2] and toad [3, 4] liver. Its degradation is measured by the formation of trichloroacetic acid soluble radioactivity during the incubation of the particles in a convenient medium [1]. These particles show several properties such as latency, and sensitiveness to pH, salt concentration, osmotic shock and other agents [4, 5]. The intraparticulate hydrolysis produces 131 I-tyrosine which is not reincorporated into proteins [6]. More recently the effects of several drugs on these particles were studied in our laboratory [7, 8].

In the present work we demonstrate that [^{131}I]-albumin digesting particles display a peculiar equilibrium density in a sucrose gradient, differing from that of other subcellular bodies, and that does not change with the age of the particles.

Material and Methods

Adult swiss albino mice weighing 30 ± 1 g were injected with 0.4 ml of farmaldehyde-treated [^{131}I]albumin (5 mg/ml and 1.5×10^7 cpm/ml) into the tail vein. The mice were sacrificed at different times after injection, and one gram of tissue from each liver was homogenized 1 : 30 (w/v) in ice-cooled 0.25 M sucrose – 0.01 M Tris acetate buffer (pH 7.4). From the homogenates, particles sedimenting at $600 \times g$ for 5 min, $27000 \times g$ for 10 min, and a final supernatant were obtained. The $27000 \times g$ particles containing most of the sedimentable radioactivity, were resuspended and they were either uniformely distributed into or layered on continous sucrose gradients. The gradients were centrifuged at $100000 \times g$ for three hours in the SW 39 rotor of the Spinco Beckman centrifuge, then eleven fractions were syphoned out from the bottom of the centrifuge tubes. The fractions were analysed for proteins [9], acid phosphatase [2], total and trichloroacetic acid soluble radioactivity; they were also incubated for the digestion test [2, 3] (1 hour, 37 °C, pH 5.0) to measure the rate of RISA intraparticulate hydrolysis. In some experiments using gradients with a superimposed sample, the fractions were centrifuged again to measure the amount and solubility of the radioactivity released by the particles.

Results

Maximal incorporation of radioactivity by liver was found 30 min after injection. Radioactivity was mostly non sedimentable at 5 min, while the opposite was true at 30 and 300 min. In the $27000 \times g$ particles recovered from the gradients, the acid soluble radioactivity existing before, and that formed during incubation for digestion test increased with time after injection (see Table).

The results obtained using gradients with uniform distribution of $27000 \times g$ particles are consigned in Fig. 1. Total radioactivity, that was widely distributed in the zone of low and medium density of the gradient at 5 min, showed a peak at the density 1.25, thirty and 300 min after injection. The acid soluble radioactivity formed during incuba, tion, and the two indexes expressing specific intraparticulate hydrolysis of RISA, showed peaks at the density 1.25 at all times. Proteins and acid phosphatase activity showed peaks at the density 1.20 and 1.21, as it was expected for a normal distribution of mitochondria and lysosomes.

A sharp distribution of acid soluble radioactivity present in $27000 \times g$ particles at the moment of sacrifice was achieved using gradients with super-

* This work was supported by a grant of the Consejo Nacional Investigaciones Científicas y Técnicas, Argentina.

Table I. Distribution of radioactivity in mouse liver homogenates, and acid soluble radioactivity in 27000×*g* particles before and after digestion test.

	Time after injection		
	5 min	30 min	300 min
Radioactivity in the organ (% of the injected)	8.8 ±2.2	15.3 ±3.1 *	9.0 ±2.0 *
Radioactivity in 600×*g* particles (% of total in liver)	5.8 ±2.9	16.7 ±3.8 *	21.1 ±4.0
Radioactivity in 27000×*g* particles (% of total in liver)	20.8 ±3.8	43.3 ±6.7 *	38.0 ±5.6
Radioactivity in supernatants (% of total in liver)	73.4 ±7.0	41.0 ±6.0	40.9 ±5.8
Acid soluble radioactivity in 27000×*g* particles before digestion test (% of total radioactivity in the gradient)	0.9 ±0.4	3.8 ±0.8 *	22.4 ±8.3 *
Acid soluble radioactivity formed during incubation by 27000×*g* particles (% of total radioactivity in the gradient)	1.3 ±0.4	2.5 ±0.7	8.3 ±2.2 *
[^{131}I]albumin hydrolyzed by 27000×*g* particles of one liver during digestion test [μg]	0.48±0.15	3.24±0.90 *	8.50±2.25 *

The data represent the average of four experiments ± standard deviation. The asterisks marking some values in the 30 min column mean statistical significance ($p<0.001$) if compared with the corresponding ones in the 5 min column. Similarly, the asterisks of the 300 min column mean statistical significance ($p<0.001$) when the values are compared with the corresponding ones in the 30 min column.

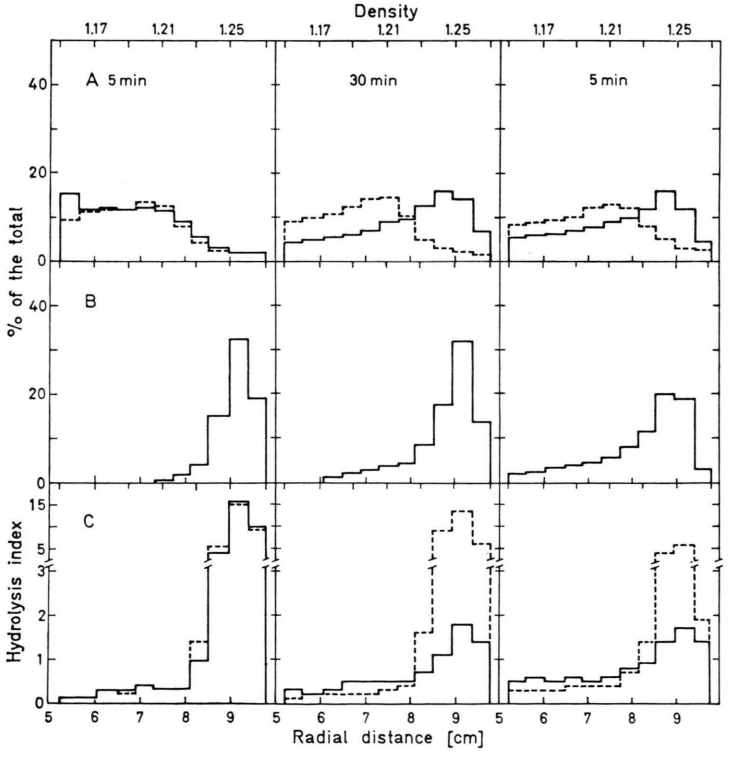

Fig. 1. Isopycnic equilibrium of 27000×*g* particles in gradients with uniform distribution of the sample obtained from liver of mice injected with [^{131}J]albumin and sacrificed 5, 30, and 300 min after injection. A: distribution of total radioactivity (solid lines); B: distribution of acid soluble radioactivity formed during incubation of the fractions for digestion test. In both A and B series of graphics the values in the abscissa represent percentages of total in the gradient; C Hydrolysis indexes. They were calculated as the % of total acid soluble radioactivity formed during incubation by the fractions/% of total acid insoluble radioactivity in the fraction at zero time of incubation (solid lines), and as the % of total soluble radioactivity formed during incubation by the fraction/total protein in the fraction (dotted lines). Before digestion test, all fractions were brought to 4 ml with sucrose solutions in 0.05 M acetate buffer (pH 5.0) to reach a final sucrose concentration of 20%.

imposed samples only (Fig. 2), since this method avoided a leakage of radioactivity from the particles during the permanence of the sample into the chambers of the gradient-forming device.

Most of the radioactivity in the fractions within the density range of 1.23 to 1.27 was recovered outside the particles. The diffused radioactivity was mostly acid soluble.

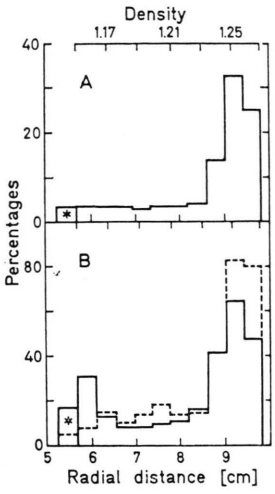

Fig. 2. Isopycnic equilibrium of $27000 \times g$ particles from liver of mice injected with [^{131}I]albumin and sacrificed 30 min after injection (gradients with superimposed sample). A: Distribution of acid soluble radioactivity in the particles before incubating for digestion test (% of total acid soluble radioactivity in the gradient). B: Radioactivity recovered in supernatants after centrifuging the fractions from the gradient at $100000 \times g$ for 20 min. Each fraction was firstly brought to 4 ml with sucrose solutions in 0.01 M Tris acetate buffer (pH 7.4) to get a final sucrose concentration of 20% with the object of ensuring particle sedimentation. Solid line: radioactivity in supernatants (% of radioactivity in the fraction). Dotted line: acid soluble radioactivity (% of the radioactivity in the supernatant of the fraction).

Discussion

In the sucrose gradients, RISA digesting particles appear as a quite homogenous population being separated from mitochondria, lysosomes and, 5 min after injection, from the bodies carrying the bulk of undegraded RISA. Their distribution strongly differs from that of Triton-related particles of rat liver [10], and the administration of RISA did not change acid phosphatase distribution, as it occurs under the treatment with the detergent. This suggests that no measurable amounts of lysosomal enzymes are necessary for the formation and performance of RISA digesting particles.

The results shown in the Table and the time-dependent shift of radioactivity distribution in the gradient suggest that an increase of labelled albumin into the digesting particles may occur with time after injection. Nevertheless, the equilibrium density values remain unchanged, no matter the age of the particles. On the contrary, the distribution of RISA changes with time after injection in a way similar tot hat shown by Jacques for endovenously injected peroxidase [11].

The fact that the acid soluble radioactivity present in the particles at the moment of sacrifice has the same distribution of that formed during the incubation is consistent with the behaviour expected for phagolysosomes bearing, in addition of intact albumin, labelled hydrolysis products formed *in vivo*.

We are now wondering if the particles that digest [^{131}I]albumin, and become equilibrited, at the density 1.25, are in essence the same that were described in rat liver as autophagic vacuoles [12] and they incorporate here the foreign protein. In this case, the methods described may constitute a starting technique to separate this kind of secondary lysosome in reasonable physiological conditions.

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