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Intra- and extramitochondrial isocitrate dehydrogenases

In the course of investigating the function of the extramitochondrial enzymes of the citric acid cycle[1], we observed that the intra- and extramitochondrial isocitrate dehydrogenase activities of rat liver do not result from identical enzymes. This report presents evidence for the heterogeneity of intra- and extramitochondrial isocitrate dehydrogenases of rat and chicken liver.

Rabbits were injected intravenously with 4–6 mg of intra- or extramitochondrial rat enzyme preparations twice a week for a period of 6 months. Serum was then taken from the rabbits, was heated at 56° for 30 min, and was stored frozen. Serum from a rabbit which had received the mitochondrial enzyme preparation was found to inhibit intramitochondrial isocitrate dehydrogenase activity but not extramitochondrial isocitrate dehydrogenase activity. Table I demonstrates the immunological differences of the two enzymes. Sera from four rabbits injected with the extramitochondrial enzyme preparation were without effect on intra- or extramitochondrial

TABLE 1

IMMUNOLOGICAL DIFFERENCES BETWEEN INTRA- AND EXTRAMITOCHONDRIAL ISOCITRATE DEHYDROGENASES OF RAT LIVER

Mitochondria of rat liver were prepared in 0.25 *M* sucrose according to the method of HOGEBOOM[2] as modified by MYERS AND SLATER[3]. The mitochondria from one rat were suspended in about 4 ml distilled water and subjected to sonic oscillation in a Raytheon Sonic Oscillator (Model DF 101, 250 W) at about 0° for 10 min. The suspension of broken mitochondria so obtained was centrifuged at 30000 rev./min in rotor number 30 of a Spinco preparative ultracentrifuge for 30 min. The supernatant contained virtually all of the mitochondrial isocitrate dehydrogenase activity. This material is called "intramitochondrial enzyme preparation". Extramitochondrial high-speed supernatant was prepared from the first supernatant remaining after centrifuging down the mitochondria by centrifugation at 30000 rev./min in rotor number 30 of the Spinco preparative ultracentrifuge for 30 min. It was dialyzed against 0.05 *M* potassium phosphate buffer at 0° for 14 h. This material is called "extramitochondrial enzyme preparation". An amount of intra- or extramitochondrial enzyme preparation which gave a change in absorbancy at 340 mμ of between 0.05 and 0.1/min in the isocitrate dehydrogenase assay[4] was incubated with about 1 ml of heated rabbit serum at 0° for 14 h. The incubation mixture was then centrifuged in rotor 40 of the Spinco preparative ultracentrifuge at 38000 rev./min for 2 h, and the supernatant so obtained was assayed for TPN-specific isocitrate dehydrogenase. The results shown were obtained with serum obtained from a rabbit which had been immunized with the intramitochondrial enzyme preparation. Sera from rabbits which had not been immunized were used as controls.

Source of enzyme activity	*Expt.*	*Percent of original isocitrate dehydrogenase activity remaining in supernatant after incubation with serum of rabbit injected with*	
		(a) intramitochondrial enzyme preparation	*(b) control*
Intramitochondrial enzyme preparation	1	4	100
	2	29	77
	3	6	86
Extramitochondrial enzyme preparation	1	100	100
	2	90	100
	3	94	77

isocitrate dehydrogenase. Although the attempts to produce antibody to the extramitochondrial enzyme have so far been unsuccessful, the results with the antiserum to the intramitochondrial enzyme are sufficient to demonstrate that the intramitochondrial enzyme differs from the extramitochondrial enzyme. Attempts to produce rabbit antibodies to the extramitochondrial isocitrate dehydrogenase of chicken liver are in progress. The major components of the intra- and the extramitochondrial isocitrate dehydrogenase activities of both rat and chicken liver have electrophoretic mobilities which differ strikingly. These electrophoretic differences are shown in Table II. In addition to a major component, the extramitochondrial enzyme activity of chicken liver also shows a minor component with isocitrate dehydrogenase activity. Visual comparison of the fluorescent bands on the starch block showed that the minor component is much weaker than the major components. Moreover, the weak band took much longer to appear than the major bands, which appeared in a matter of minutes. It is concluded that the isocitrate dehydrogenase of mitochondria differs from that found extramitochondrially.

The heterogeneity of intra- and extramitochondrial malate dehydrogenases has also been demonstrated by a number of authors[6–8]. The occurrence of enzymes in different compartments of the cell which catalyze the same reaction, but which differ

TABLE II

ELECTROPHORESIS OF INTRA- AND EXTRAMITOCHONDRIAL LIVER PREPARATIONS ON STARCH BLOCKS[5]

The rat-liver preparations were made as described in Table I. The chicken-liver preparations were made by the same method. Intra- and extramitochondrial preparations from the same species were run side by side on the same block. The suspending medium for the starch was 0.05 *M* Tris–HCl buffer (pH 8.10). The electrophoresis was run at 4 V/cm for 17.5 h, the current was switched off, the starch block was blotted thoroughly, and developing agent was sprayed along the block from a vaporizer. The developing reagent consisted of a solution containing 20 m*M* trisodium isocitrate (synthetic), 5 m*M* $MnCl_2$, and 2 mg/ml TPN^+, adjusted to pH 7.5 with Tris. After allowing the reaction to proceed for a suitable interval, the block was viewed under ultraviolet light, and the fluorescence due to TPNH formed in the isocitrate dehydrogenase reaction was marked. The results are expressed as the mean distance in cm that each band migrated towards the cathode from the line of application. The words "strong" and "faint" refer to a visual estimate of the fluorescence intensity.

	Distance of fluorescent band from origin in cm	
	Intramitochondrial enzyme preparation	*Extramitochondrial enzyme preparation*
Rat	—	0.5 strong
	6.8 strong	—
Chicken	—	2.1 strong
	9.7 strong	9.0 faint

from one another in physical, chemical, and kinetic behavior is presumably a reflection of the different properties which each particular enzyme must have in order to function efficiently in the environment of its cellular compartment.

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