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Concerning the Mechanism of Succinate Oxidation in Albino Rat Liver

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Experiments with deuterated succinate (2,3-dideuteriumbutanediacid) prove that succinate is oxidized via the peroxide pathway. After the addition of deuterated succinate to liver centrifugate, heavy water is formed as a result of its dehydration.

Key Words: *deuterated succinate; heavy water; liver pellet; fumarate*

In investigating the role of the hydrogen peroxide—catalase system in tissue respiration, we hypothesized that succinate oxidation is associated with the formation of peroxides [1,2]. Dehydration of succinate in the process of tissue respiration is catalyzed by flavoprotein oxidases, which display peroxidase activity under aerobic conditions [9]. Consequently, succinate can be directly oxidized by molecular oxygen with the participation of flavoprotein enzymes [4,7,9]. To clarify this issue, experiments with deuterated succinate (DS) were performed, which allowed us to trace the fate of the succinate hydrogen in the α-position relative to the carboxyl group.

MATERIALS AND METHODS

Deuterated succinate was synthesized from sodium maleate with mercury as a catalyst. Sodium maleate (1 g) was dissolved in 4.2 ml 99% heavy water and vigorously shaken with sodium amalgam until sodium succinate crystals formed. Discoloration of potassium permanganate indicated the end of the reaction. The crystals were filtered out, dissolved in water (5 ml), and neutralized with HCl. Deuterated succinic acid was extracted with ether. The ether was distilled, and the residue dried and tested for purity. Its melting point proved to be 183°C. DS (2,3-dideuteriumbutanediacid) thus obtained was used in the experiments.

Livers (3 g) of intact outbred albino rats were homogenized in the cold in phosphate buffer (6 ml, pH 7.3) and centrifuged at 15,000 g, after which DS was added. Incubation was carried out for 4 h. Succinate and DS in phosphate buffer were used as controls.

After the incubation, proteins were precipitated, and the content of heavy water was determined in transparent centrifugate obtained by distillation under low pressure in special quartz equipment. The sensitivity of the method was ±0.04 mol/dl.

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TABLE 1. Content of Heavy Water and Fumaric Acid in Samples after a 4-h Incubation

Sample	Heavy water content (%)±2%	Fumaric acid content (mmol/liter)±7%
Control (succinate with liver centrifugate)	0.04	0
Control (DS in phosphate buffer)	0.04	0
DS with liver centrifugate	0.34	0.17
DS with liver centrifugate under anaerobic conditions	0.05	0

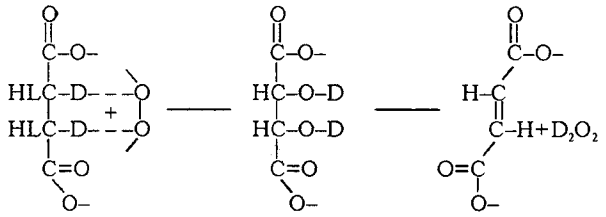
Since succinate does not form an azeotropic compound with water, DS was not detected in distilled water.

Experimental protocol: DS in phosphate buffer (pH 7.3) served as the control; 90 mg DS were added to 2 ml liver centrifugate (succinate was added to the other sample). Incubation was carried out at 37°C under aerobic and anaerobic conditions. The content of fumaric acid in each sample was determined in an IKS-14A spectrophotometer according to the double-beam compensatory scheme. Fumaric acid has a specific absorbance spectrum with a characteristic set of nonoverlapping bands permitting its identification by spectral curve analysis. Fumaric acid was identified in the reaction mixture by the presence of the 1270 cm^{-1} high absorbance band.

RESULTS

From an analysis of the results summarized in Table 1 it can be concluded that heavy water is synthesized during the incubation of DS with liver centrifugate under aerobic conditions as a result of dehydration of DS.

All samples were tested for the presence of fumaric acid (1270 cm^{-1} absorbance band [3]). This band was detected only in the spectrum of the DS-liver centrifugate mixture after incubation under aerobic conditions. The discovery of heavy water and fumaric acid led us to postulate the following mechanism of succinate dehydration in a living cell. Oxygen reacts with polarized hydrogen of maleic acid in the α-position (the reaction is catalyzed by enzymes) with the formation of succinate peroxide. This compound is attacked by flavoproteins with peroxidase activity, resulting in the formation of fumaric acid and hydrogen peroxide. The reaction probably proceeds according to the following scheme:



Hydrogen peroxide is decomposed by catalase with the formation of active radicals which, it is thought, transfer their energy for the synthesis of peptide bonds and phosphoanhydrides [2].

This hypothesis agrees with the evidence that the removal of oxygen from the incubation medium of mitochondria under cyanide blockade results in the cessation of phosphorylation [5,6]. Mitochondrial respiration is restored after the addition of lipid peroxides. Moreover, it has been demonstrated that inhibitors of peroxidase lower the rate of coupled oxidative phosphorylation [5]. Consequently, peroxides play an important role in tissue respiration, specifically in the process of succinate dehydration.

Thus, with the use of DS we have proved that in albino rat liver succinate is oxidized via the peroxide pathway.

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