OXIDATION OF EXOGENOUS NICOTINAMIDE DINUCLEOTIDE BY ISOLATED MITOCHONDRIA

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ABSTRACT

Oxidation of Exogenous NADH by Isolated Mitochondria.

Preparations of rat liver mitochondria oxidized added NADH by the respiratory chain at an average rate of 2.2 nmoles NADH oxidized/min x mg mitochondrial protein, although NADH generated internally from glutamate + malate in the presence of ADP + Pi was oxidized at a rate of 120 nmoles NADH oxidized/min x mg mitochondrial protein. Succinate oxidase activity of the isolated mitochondria was not affected by 1.67 mM oxaloacetate in the media. If the liver mitochondria are damaged by freeze-thawing in hypotonic media, 0.3 mM oxaloacetate inhibits the succinate oxidase activity 50%. Isolated rat liver mitochondria rapidly oxidized NADH when oxaloacetate was added, presumably by the m-malate dehydrogenase at a rate of 2,400 nmoles NADH oxidized/min x mg mitochondrial protein. Deoxycholate solubilization of the mitochondria increased this activity only slightly to 2,900 nmoles NADH oxidized/min x mg mitochondrial protein. The m-malate dehydrogenase activity of the intact rat liver mitochondria was not removed by washing or effected by phosphate concentration. It was slightly inhibited by adenine nucleotides and Tris. Thus, the m-malate dehydrogenase of rat liver is apparently not primarily latent and is probably located either in or upon the inner mitochondrial membrane with an outward orientation.

Isolated beef heart mitochondria also rapidly oxidized added NADH in the presence of oxaloacetate by m-malate dehydrogenase at a rate of 1,115 nmoles NADH oxidized/min x mg mitochondrial protein.

However, solubilization of the heart mitochondria increased the activity twelve-fold to a rate of 14,230 nmoles NADH oxidized/min x mg mitochondrial protein. Phosphate concentration had no effect on the original or solubilized activity, but ADP, ATP, KCl, and sucrose were inhibitory. The activity was not washed away.

The oxidation rate of added NADH by the respiratory chain of isolated beef heart mitochondria was 50-150 nmoles NADH oxidized/min x mg mitochondrial protein. This NADH oxidase activity was 30% of that measured for NADH + pyruvate + malate oxidation in the presence of ADP and Pi and 30% of that measured in cholate solubilized mitochondria. The NADH oxidase activity was independent of endogenous substrate, pyruvate + malate, or succinate oxidation and of ADP, Pi, and ATP. The NADH oxidase was more sensitive to rotenone, antimycin, and cyanide inhibition than was the oxidation of pyruvate + malate. It was concluded that NADH oxidase activity of isolated beef heart mitochondria was likely due to damaged mitochondria in the population. The malate dehydrogenase activity of isolated heart mitochondria is then attributable to exposure by damage with some activity lost to the media and some remaining bound to the inner membrane.

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