Effects of substrates on the swelling and loss of pyridine nucleotides from rat-liver mitochondria*

In the course of an investigation into the mechanism of the loss of pyridine nucleotides from isolated rat-liver mitochondria, it was observed that a rapid leakage of the coenzymes from the particles occurs in the presence of inorganic phosphate. Accompanying the release of the pyridine nucleotides, the mitochondria begin to swell. Coincident with the leakage and swelling, we have found that the pyridine nucleotides are destroyed. A similar relationship is observed with other agents which induce either of the three phenomena, leakage, swelling or destruction. Since depletion[1] and swelling[2] of the mitochondria fail to occur under anaerobic conditions, it would appear that these two processes are somehow dependent on the oxidative reactions of the mitochondria. This possibility is further increased by the observation that a rapid oxidation of the intra-mitochondrial reduced pyridine nucleotides takes place during the depletion incubation and only the oxidized forms of the pyridine nucleotides leak out of the mitochondria.

We have therefore examined the effects of various substrates on the leakage of the pyridine nucleotides and the swelling of mitochondria. Mitochondria from rat liver were prepared and suspended in 0.25 *M* sucrose. To 2 ml mitochondria (equivalent to 1 g of original tissue) was added 0.5 ml 0.25 *M* sucrose containing 0.1 *M* potassium phosphate, pH 7.5, and 0.05 *M* nicotinamide. Substrates were used in a 20 m*M* final concentration. The mixtures were incubated at 30° with constant shaking and at the specified time immediately returned to ice. 0.05 ml of the suspension was added to 2.95 ml 0.25 *M* sucrose containing 0.05 *M* ethylenediaminetetraacetate, and the decrease in absorbance at 520 mμ was taken as a measure of swelling. To determine the leakage of the pyridine nucleotides, the remaining mitochondrial suspension was rapidly centrifuged at 0° and the total oxidized pyridine nucleotides were determined in a 5 % trichloroacetic acid extract of the resulting supernatant fluid by developing the methyl ethyl ketone fluorescence[3].

Fig. 1a shows that the leakage of pyridine nucleotides from mitochondria incubated in the presence of phosphate is almost completely inhibited by succinate. Malonate effectively reverses the inhibition while malonate in the absence of succinate results in a slight stimulation of the leakage. In marked contrast to the observations of previous investigators[4,5], Fig. 1b shows that under these conditions succinate also inhibits the phosphate-induced swelling of the mitochondria and this too is reversed by malonate. Again, malonate alone results in a slight stimulation of the phosphate swelling. Similar effects are observed with isocitrate, glutamate, and β-hydroxybutyrate. All of these substrates, however, are less effective in preventing both leakage and swelling; β-hydroxybutyrate is the least effective.

Various attempts have been made to relate the above-described effects of succinate on mitochondrial swelling with the opposite observations of previous investigators[4,5]. Omission of nicotinamide has no significant effect on the swelling or leakage but prevents the destruction of the oxidized pyridine nucleotides. Isolation of the mitochondria in 0.44 *M* sucrose and resuspending in 0.3 *M* sucrose also has no significant effect on the succinate inhibition as long as the reaction mixture consisted of

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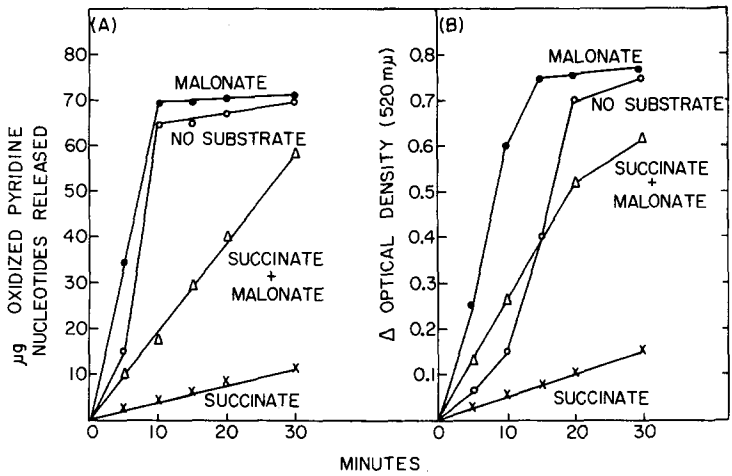

Fig. 1. Effects of succinate and malonate on the phosphate-induced leakage of pyridine nucleotides and swelling of rat-liver mitochondria. Each tube contained mitochondria equivalent to 1 g original tissue suspended in 2.5 ml 0.25 *M* sucrose containing 20 m*M* potassium phosphate, pH 7.5. Substrates were added in a 20 m*M* final concentration. Incubations were carried out with shaking at 30°. (A) represents the pyridine nucleotides present in the medium after removal of the mitochondria at various time intervals by centrifugation; (B) represents the change in absorbance at 520 mμ of 0.05 ml of the incubation mixture resuspended in 2.95 ml of 0.25 *M* sucrose containing 0.05 m*M* ethylenediaminetetraacetate.

at least 2 ml of mitochondrial suspension. Addition of 25 m*M* tris(hydroxymethyl)-aminomethane to the incubation medium results in a slight inhibition of the leakage and swelling.

It would appear that this difference is due, probably, to the concentration of mitochondrial particles in the incubation mixture. At high concentrations of particles, succinate effectively inhibits the swelling induced by inorganic phosphate. Reducing the concentration approximately 40 times results in a stimulation of this form of swelling. Although the exact nature of the leakage and swelling is obscure, the results of this study suggest that under these conditions succinate maintains the pyridine nucleotides as well as the respiratory chain in the reduced state[6], thereby simulating the anaerobic inhibition of swelling[2] as well as the inhibition of leakage and depletion of the pyridine nucleotides.

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