INHIBITION OF SUCCINATE OXIDATION IN BEEF HEART MITOCHONDRIA BY DERIVATIVES OF PYRIDINE ADENINE DINUCLEOTIDE

By

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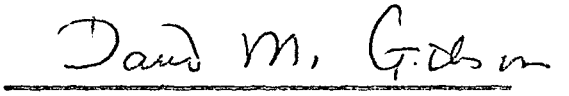
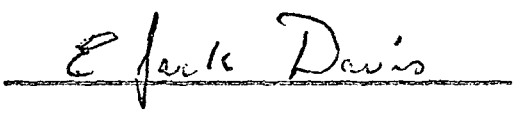
Dr. David M. Gibson 

Dr. E. Jack Davis 

ABSTRACT

The inhibition of succinate oxidation by nicotinamide adenine dinucleotide (NAD^+) has been observed in sonicated mitochondria and submitochondrial preparations. Two mechanisms for the inhibition by NAD^+ have been suggested. The first postulates that NAD^+ causes the production of oxaloacetate, an inhibitor of succinate oxidase, from succinate via fumarate and L-malate. The second postulates that NAD^+ or its derivatives attaches directly to succinate dehydrogenase to cause inhibition. With disrupted beef-heart mitochondria, it was noticed that low levels of NADH (reduced NAD^+) also inhibit succinate oxidation. The inhibition appeared after a lag phase. It was thought that the inhibition by NADH was actually caused by NAD^+, which stimulated the production of oxaloacetate, a potent inhibitor of succinate oxidation. Thus, an investigation of the nature of NADH inhibition of succinate oxidation led to experiments designed to evaluate the inhibition caused by NAD^+ analogues.

All dinucleotides tested, NAD^+, 3-acetylpyridine adenine dinucleotide (3-$AcPAD^+$), 3-acetylpyridine deaminoadenine dinucleotide (3-$AcPDeAD^+$), nicotinamide adenine dinucleotide phosphate ($NADP^+$), NADH (reduced NAD^+), NADPH (reduced $NADP^+$), with the exception of NADPH, inhibited succinate oxidation. The inhibition had a lag period and increased with time. However, the increase in inhibition with time by $NADP^+$ came to an early steady state (percent inhibition did not change), whereas the inhibition by the

other dinucleotides continuously increased.

L-malate, which stimulated the production of oxaloacetate in the presence of NAD^+ and its analogues enhanced inhibition, and compounds (glutamate, rotenone, NADH plus rotenone, meso-tartrate) which removed oxaloacetate or stopped its production prevented or reversed inhibition. The oxidized dinucleotide did not inhibit succinate oxidation in purified electron transport particles. A supernatant fraction restored the inhibitory ability of NAD^+.

Differential spectra and oxidation-reduction of the dinucleotides at absorption maxima were recorded in the presence of succinate and L-malate. The appearance of the reduced form of the NAD^+-analogues was positively correlated with the percent inhibition of succinate oxidation. The reduced analogues accumulated because they were not rapidly oxidized by the respiratory chain, whereas NADH did not accumulate because it was rapidly reoxidized.

The data are consistent with the postulate that oxaloacetate is the direct inhibitor of succinate oxidation, and that NAD^+ stimulates the production of oxaloacetate. No evidence indicates that NAD^+ directly inhibits succinate dehydrogenation.

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