CONTROL OF NADH OXIDATION BY ADP IN MEMBRANES FROM MYCOPLASMA LAIDLAWII[1]

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Mycoplasma cells are particularly well suited for studies of membranes because of the absence of a cell wall and, therefore, the ease of effecting lysis by osmotic shock. The use of this mild technique has indicated that the major part of the NADH oxidase activity is localized in the membrane fraction of Mycoplasma laidlawii (Pollack et al., 1965a, 1965b).

In a study of the terminal respiratory pathway of M. laidlawii, it has been shown that the oxidation of NADH is inhibited by adenine nucleotides (Stopkie and Weber, 1967). The inhibition or activation of enzyme catalyzed reactions by purine and pyrimidine nucleotides has been extensively reviewed (Monod et al., 1963, 1965; Stadtman, 1966). This communication reports the differential inhibition by ADP of the oxidation of NADH in various subcellular fractions of M. laidlawii, strain A.

MATERIALS AND METHODS

M. laidlawii was grown in a modification of a medium previously described (Weber and Kinsky, 1965). Cells were grown to a concentration of 1.8×10^9 cells/ml and harvested at 9,000 x g for 15 minutes at 4° C. The cells were washed with buffer containing 0.15 M NaCl and 0.05 M Tris-HCl, pH 8.1 (Pollack et al., 1965b), and were suspended at 37° C in 7.5 mM NaCl and 2.5 mM Tris-HCl, pH 8.1, at a ratio of 1 g wet weight of cells to 1.0 ml

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of buffer. The cells were lysed by slowly dripping the suspension into the same buffer (amount to achieve a 10% (w/v) cell suspension) with constant stirring over a 5 minute period. Remaining whole cells and debris were removed by centrifugation at 9,000 x g for 10 minutes. The membrane fraction was obtained by centrifugation at 37,000 x g, and the supernatant fluid centrifuged at 105,000 x g to obtain the particulate and soluble fractions. The membrane and particulate fractions were washed twice with the buffer used for lysis of the cells, and the soluble fraction was recentrifuged twice.

The oxidation of NADH was measured following the change in absorbancy at 340 mμ per minute in a Zeiss PMQII spectrophotometer at 30° C.

In experiments where solubilization of membrane fragments was performed, sonic vibration was used for varied time periods in a 10 kc Raytheon Sonic Oscillator tuned to maximum output at 1.25 amp. Criterion for solubilization was careful removal of the supernatant fluid after centrifugation at 105,000 x g for 3 hours and recentrifugation for an additional 3 hours.

RESULTS AND DISCUSSION

As observed in Fig. 1, the inhibition of the membrane-associated NADH oxidase is a hyperbolic function of the concentration of ADP approaching the 85% level at high inhibitor concentration with 50% inhibition at approximately 8 mM. In a number of experiments it was found that this concentration of ADP would not inhibit by 50%, and it has been subsequently found that it was due to breakdown of ADP on storage. Although not indicated here, in addition to ADP, ATP and AMP are also inhibitory, but at significantly higher concentrations and appear to inhibit by different mechanisms. This is unlike

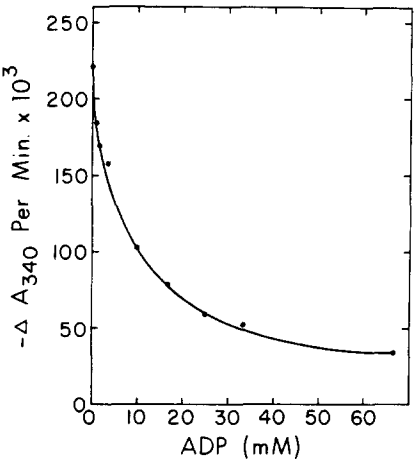

Fig. 1. Effect of varying concentrations of ADP on the oxidation of NADH. The reaction mixtures contained 0.5 ml of M/1 PO_4 buffer, pH 6.8, 0.069 mg of membrane protein, 5'-ADP as indicated, and deionized H_2O to a total volume of 3 ml. The ADP was incubated with the membranes for 3 minutes, and the reactions were started by addition of 0.44 μmole of NADH.

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the pigeon heart mitochondrial system described by Blanchaer and Griffith (1966) and Griffith and Blanchaer (1967) where they found that ATP consistently produced the same reduction in rate with similar kinetics as an equimolar amount of ADP. The pyrimidine nucleotides CMP, CDP, and TMP showed 20-30% inhibition at concentrations 10 times that required to achieve 50% inhibition by ADP. In contrast to the membrane fraction of M. laidlawii, a particulate NADH oxidase from Mycobacterium tuberculosis was shown to be activated by AMP (Worcel and Goldman, 1964; Worcel et al., 1965).

The results of the effect of ADP on NADH oxidase activity by the subcellular constituents of the cells are shown in Table I.

As indicated, the NADH oxidase associated with the soluble fraction is not inhibited by ADP, whereas the particulate fraction has a value between that of the membrane and the soluble. In experiments to be reported elsewhere, it

TABLE I

COMPARATIVE INHIBITION OF NADH OXIDASE ACTIVITY BY ADP IN MYCOPLASMA CELL FRACTIONS

Cell fraction	$-\Delta A_{340}$/minute/mg protein		% inhibition by 9 mM ADP
	No ADP	9 mM ADP	
MEMBRANE (37,000 x g pellet)	1.59	.90	43
PARTICULATE (105,000 x g pellet)	.045	.034	24
SOLUBLE (105,000 x g supernate)	.10	.105	0

The reaction mixtures, in a final volume of 3.0 ml, contained 0.5 ml of M/1 PO_4 buffer, pH 7.0, 0.52 μmole of NADH, 27 μmoles of ADP, and protein for individual reactions as follows: 0.07 mg of membranes, 1.96 mg of particulate fraction, and 0.99 mg of soluble fraction. ADP was preincubated with fractions for 3 minutes at 30° C before reactions were started with NADH.

has been found that as the culture ages, the soluble fraction acquires ADP sensitivity.

To determine whether the soluble fraction was a membrane breakdown product caused by the method of lysis, a comparison was made between the in situ soluble fraction and membranes solubilized by treatment with sonic oscillation. Fig. 2A shows a 70% increase in NADH oxidase activity of the whole membrane preparation with increasing time of sonication, and Fig. 2B shows the concomitant appearance of activity in the supernatant fluid (solubilized fraction). In contrast to the in situ soluble fraction, this solubilized membrane fraction retained ADP sensitivity, which was actually increased by 15-20% over that of the intact membrane. This finding is in partial agreement with Griffith and Blanchaer (1967) who found an increased rate of NADH

oxidation in sonicated mitochondria, but the percentage of inhibition by ATP and ADP remained nearly constant after treatment with sonic oscillation. Our data suggest that the in situ soluble fraction is indeed different from an artificially produced soluble fraction, especially since sonication is presumably a more drastic procedure than osmotic lysis.

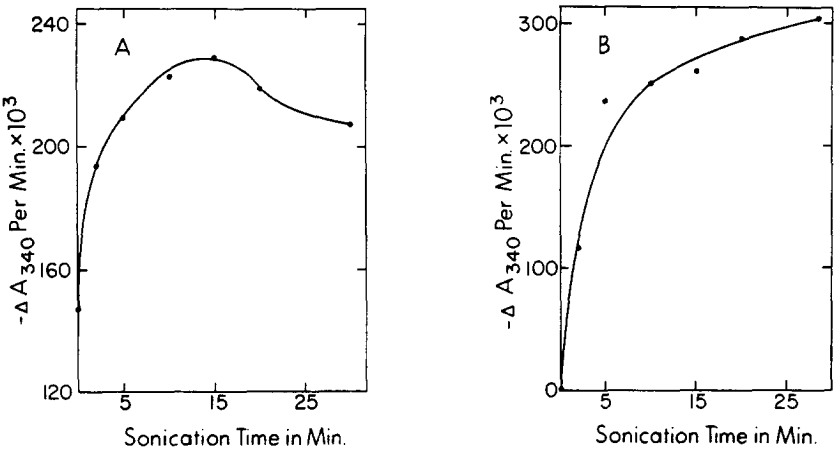

Fig. 2A. Effect of time of sonication on the oxidation of NADH catalyzed by the membrane fraction. Aliquots were removed from the sonicator at designated times and placed at 0° C until assayed. The reaction mixtures contained 0.5 ml of M/1 PO_4 buffer, pH 6.8, 0.06 mg of membrane protein, and deionized H_2O to a total volume of 3 ml. The reactions were started by addition of 0.4 µmole of NADH.

2B. Effect of time of sonication on solubilization of the membrane fraction. The membrane fractions were centrifuged at 105,000 x g for 3 hours. The supernatant fluid was removed and recentrifuged for an additional 3 hours, and the resultant soluble fraction was carefully removed and assayed.

Centrifugation of the sonicated crude membrane preparation at 105,000 x g yielded a membranous pellet of presumably smaller fragments and a supernatant fluid containing the solubilized membranes. As indicated in Fig. 3, whereas the specific activity of the solubilized membranes remained constant from the shortest time interval of sonication to the longest (30 minutes), the pellet (membranes) increased in activity.

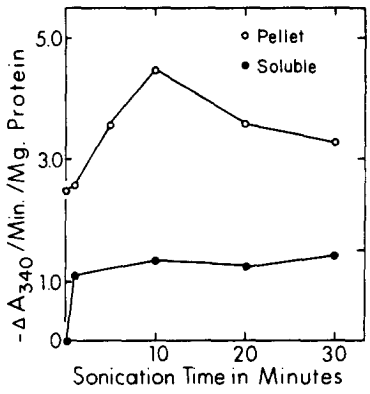

Fig. 3. Effect of time of sonication on NADH oxidase activity of pelletable membrane fragments and solubilized membranes. Aliquots were removed at various time intervals and centrifuged at 105,000 x g, yielding the membranous pellet and supernatant soluble fraction. The reaction mixtures contained 0.5 ml of M/1 PO_4 buffer, pH 6.8, variable protein, and deionized H_2O to a total volume of 3 ml. Reactions were started with 0.45 μmole of NADH.

SUMMARY

The oxidation of NADH catalyzed by fractions of osmotically lysed M. laidlawii, strain A, has been examined, and the data suggest that this enzymatic reaction, associated with the membrane fraction, is controlled most specifically by ADP. This nucleotide, however, does not effect the oxidase in the in situ soluble fraction. Solubilization of the membrane retains and enhances the control of NADH oxidase by ADP.

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