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Ca^{2+} STIMULATION OF THE EXTERNAL NADH DEHYDROGENASE IN JERUSALEM ARTICHOKE (HELIANTHUS TUBEROSUM) MITOCHONDRIA

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The stimulation of the external NADH dehydrogenase in Jerusalem artichoke (Helianthus tuberosum) mitochondria by Ca^{2+} has been investigated. By varying the extramitochondrial $[Ca^{2+}]$ between 10^{-7}M and 10^{-3}M using a Ca^{2+}/EGTA buffer system we find a considerable increase in NADH oxidation at micromolar free Ca^{2+} concentrations. The NADH induced mitochondrial membrane potential is reduced if the extramitochondrial Ca^{2+} is decreased to 10^{-7}M. When $[Ca^{2+}]$ is increased to about 10^{-6}M the membrane potential is restored. The results indicate that Ca^{2+} may be a modulator of the mitochondrial external NADH dehydrogenase in vivo.

The role of an increase in the cytosolic free Ca^{2+} concentration from 10^{-7}M to 10^{-6}M or 10^{-5}M as a trigger of various activities in mammalian cells is well established (1-3). Recently it has been demonstrated that large algae have a low cytosolic $[Ca^{2+}]$ in resting conditions (4). Furthermore, a Ca^{2+} dependent regulation of NAD^{+} kinase (5,6) and microsomal Ca^{2+}-Mg^{2+} ATPase (7) have also been described for higher plants. These results suggest that the cytosolic Ca^{2+} may play a regulatory role in plant tissues as well.

In contrast to mammalian tissues, in plant cells cytosolic NADH may enter the mitochondrial respiratory chain directly by way of an externally located NADH dehydrogenase (8). This enzyme is located on the outer surface of the inner mitochondrial membrane, donates reducing equivalents into the b-c_1 complex of the respiratory chain (8) and appears to be inhibited by Ca^{2+} chelators (9).

In this report we have examined the question of Ca^{2+} control of the external NADH dehydrogenase in an attempt to determine the $[Ca^{2+}]$ at which it is activated.

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Methods and Materials

Jerusalem artichoke (Helianthus tuberosum) mitochondria were isolated as described in (10). The basal experimental medium contained 0.22M manitol, 40 mM KCl, 7 mM Hepes pH 7.2 (KOH). Oxygen consumption was measured with a Clark type oxygen electrode.

For measurements of membrane potentials, tetraphenylphosphonium (TPP^+) selective polyvinylchloride membranes were prepared as described in (11) and were glued onto used Radiometer F2112 Ca^{2+} selective electrode tubes after removal of the Ca^{2+} selective membrane. The incubation medium was connected through an Agar/KCl bridge to a reference KCl electrode.

The free Ca^{2+} concentration of the medium in the presence of the EGTA/Ca^{2+} buffer system was calculated using the reiterative method of Portzehl et al. (12) using the stability constants as described in (12).

The Ca^{2+} content in the incubation medium under various experimental conditions was measured in the presence of 100 μM arsenazo III using the wavelength pair 665-685 (13) in an Aminco DW_2 spectrophotometer. The arsenazo III was purified as in (13).

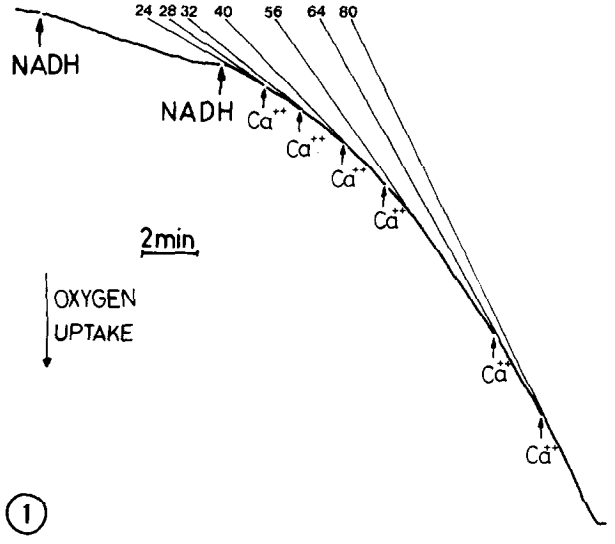
Results

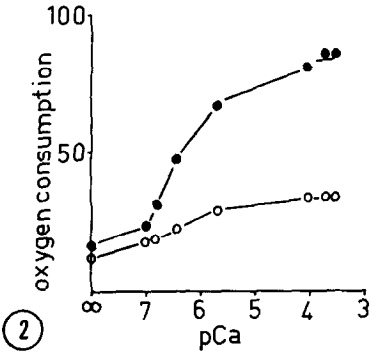
The Ca^{2+} concentration of the external medium in the presence of different batches of mitochondria is of the order of 400-700 μM as measured by adding pulses of EGTA (10 μM) in the presence of arsenazo III (not shown). This Ca^{2+} was taken into account upon calculation of the pCa in the experiments described below.

Fig.1 shows that when the Ca^{2+} activity of the incubation medium is increased stepwise from a pCa of 7.4 to about 4 NADH oxidation, via the externally located NADH dehydrogenase, is enhanced. An initial small NADH pulse was added in order to obtain a linear trace. Although a small lag period is seen after each Ca^{2+} addition, the subsequent rate of respiration attained at a certain [Ca^{2+}] is linear until anaerobiosis.

Fig.2 shows NADH respiration as a function of pCa in the presence and absence of an uncoupler, FCCP. The respiration is increased when the free Ca^{2+} concentration rises above 10^{-7}M and is near its maximal value at 10^{-6}M[Ca^{2+}]. The increase in respiration induced by FCCP is considerably enhanced at high [Ca^{2+}] (10^{-6}M).

Fig.3(a) depicts membrane potential measurements using a TPP^+ specific electrode. The addition of NADH, in the presence of 1 μM TPP^+, results in a rapid uptake of TPP^+ corresponding to the establishment of a membrane





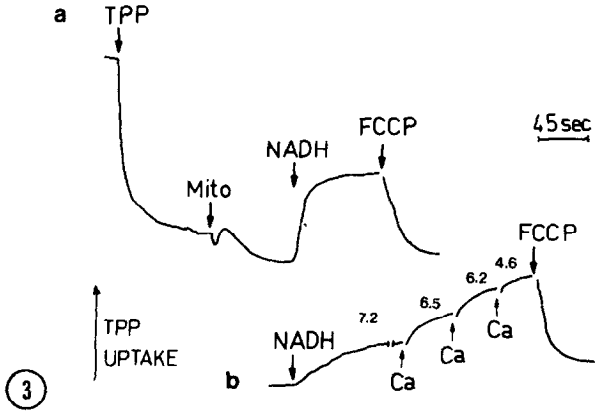


Fig.1. Effect of Ca^{2+} on the NADH linked oxygen consumption of Jerusalem artichoke mitochondria.
Mitochondria 0.55 mg protein/ml were suspended into the basal medium containing additionally 1mM EGTA and 1mM $MgCl_2$. The endogenous Ca^{2+} was 470 µM as measured with arsenazo III[2]. Additions: 50 µM and 750 µM NADH (NADH) and 100 µM Ca^{2+} (small arrows). The values above the trace denote the rate of respiration (nmol O_2/mg protein per min.).

Fig.2. Ca^{2+} dependency of NADH oxidation.
Conditions as in Fig.1. The oxygen consumption (nmol O_2/mg protein per min) measured in the presence (●) or absence (○) of 0.6 µM FCCP as a function of pCa. pCa ∞ denotes respiration in the presence of 5 mM EGTA. Endogenous Ca^{2+} is 700 µM.

Fig.3. Effect of Ca^{2+} on the membrane potential of Jerusalem artichoke mitochondria during NADH respiration.
Conditions as in Fig.1.
(a) Additions were made of 1 µM TPP^+, 0.5 mg mitochondrial protein (Mito), 0.75 mM NADH, 0.6 µM FCCP.
(b) Contained 1 mm EGTA and 1 mM $MgCl_2$, 1 µM TPP^+ and 0.5 mg mitochondria. Additions were made of 0.75 mM NADH, and various Ca^{2+} concentrations (Ca) (pCa values indicated above the trace). The endogenous Ca^{2+} was 620 µM.

potential which is rapidly depolarised by 0.6 μM FCCP. If the membrane potential is monitored under similar conditions to Fig.2, it can be seen that there is a considerable decrease in the TPP^+ response to NADH when the Ca^{2+} concentration is reduced to 10^{-7}M (Fig.3(b)). This suggests that NADH respiration is insufficient in the generation of an optimal membrane potential at low [Ca^{2+}]. Increasing the external free [Ca^{2+}] to about 10^{-6}M restores the membrane potential to that of the control trace. The magnitude of membrane potential, calculated using a value for the internal matrix space of 1 μl/mg protein, is about 150 mv at 10^{-7}M as compared to a maximal value of 200 mv at higher Ca^{2+} concentrations. It should be noted that an activity coefficient of 1 was assumed in the calculation of the membrane potentials which is probably not the case. Therefore the values represent overestimates.

Discussion

The results of the present study suggest that Ca^{2+} appears to increase the activity of the mitochondrial external NADH dehydrogenase. Since a high cation medium was used, the stimulation observed was probably not due to any charge screening effect (9). The finding that NADH respiration is enhanced considerably at micromolar external Ca^{2+} concentrations is of interest since in animal tissues various functions such as muscle contraction (14), secretion (15) and various enzymatic activities (3) are activated between a pCa of 7 and 6-5. There are also several recent findings indicating that in plants Ca^{2+} may be a trigger of various activities (5-7).

During active metabolism in plant tissues the main pathway for shunting reducing equivalents from the cytosol to the respiratory chain is probably the mitochondrial external NADH dehydrogenase. Our finding that NADH respiration is considerably reduced and the mitochondria are unable to generate an optimal membrane potential at 10^{-7}M Ca^{2+} or less suggests that the activity of the enzyme is rate-limiting. The small increase in respiration by FCCP is in agreement with this. Thus, it does not seem unreasonable to suggest that the external NADH dehydrogenase is an important

control point in plant cell metabolism and is modulated by the cytosolic $[Ca^{2+}]$. The possible involvement of calmodulin in this process is at present uncertain. However, the lack of sensitivity to low concentrations of phenothiazines (unpublished results) would tend to discount this idea, the simplest interpretation being that Ca^{2+} binds directly to the external NADH dehydrogenase to activate it.

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References

1. Borle, A.B. (1981). Rev.Physiol.Biochem.Pharmacol. 90, 13-153.
2. Carafoli, E. and Crompton, M. (1978). Curr.Top.Membr.Transp. 10, 151-216.
3. Rasmussen, H. and Goodman, D.B.P. (1977). Physiol.Rev. 57, 421-509.
4. Williamson, R.E. and Ashley, C.C. (1982). Nature 296, 647-651.
5. Anderson, J.M., Charbonneau, H., Jones, H.P., McCann, R.P. and Cormier, M.J. (1980). Biochemistry 19, 3113-3120.
6. Dieter, P. and Marmé, D. (1980). Cell Calcium 1, 279-286.
7. Dieter, P. and Marmé, D. (1980). Proc.Natl.Acad.Sci. 77, 7311-7314.
8. Douce, R., Mannella, C.A. and Bonner, W.D. Jr. (1973). Biochim.Biophys. Acta. 292, 105-116.
9. Moller, I.M., Johnston, S.P. and Palmer, J.M. (1981). Biochem.J. 194, 487-495.
10. Moore, A.L. and Proudlove, M.O. (1982). In "Isolation of membranes and organelles from plant cells" (J.L. Hall and A.L. Moore, eds.). Academic Press. In press.
11. Kamo, N., Maratsugo, M., Hongoh, R. and Kobatake, Y. (1979). J.Membr.Biol. 49, 105-121.
12. Portzehl, H., Caldwell, P.C. and Ruegg, J.C. (1964). Biochim.Biophys.Acta. 79, 581-591.
13. Scarpa, A. (1979). Methods Enzymol. 56, 301-338.
14. Endo, M. (1977). Physiol.Rev. 57, 71-108.
15. Douglas, W.W. (1974). Biochem.Soc.Symp. 39, 1-28.