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Short Communication

A Transplasmamembrane Electron Transport System in Maize Roots

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ABSTRACT

Ferricyanide is actively reduced by intact maize (*Zea mays* L., var XL 342) roots. This reduction is salt and temperature dependent, is stimulated by fusicoccin, and is accompanied by decrease of external pH. In anaerobic conditions, ferricyanide partially restores fusicoccin-induced proton extrusion. A salt-, temperature-, and pH-dependent cyanide-insensitive NADH-ferricyanide oxidoreductase activity can be demonstrated in microsomes isolated from the same plant tissue. This evidence supports the hypothesis, as proposed by Craig and Crane (1982 Plant Physiol 67: S-558, S-835), that the ferricyanide reduction is carried out by a transmembrane NADH dehydrogenase.

Direct coupling of oxidation-reduction reactions to ion transport without involvement of ATP in mitochondria has led to consideration of analogous functions in plasma membranes, where transport is a major function (13).

A transmembrane electrochemical proton gradient can be established either by a plasmalemma ATPase and the hydrolysis of ATP (7, 8) or by a redox loop consisting of an alternating sequence of proton and electron carriers (14) without intervention of ATP.

The direct use of oxidation-reduction reactions to drive transport of ions or aminoacids in mitochondrial and plasma membranes of bacteria has been described (9); a similar function was suggested in the plasmalemma of plant cells by Lin and Craig (4, 5, 11, 12).

The results presented in this paper confirm the existence of a NADH-ferricyanide (O_2) electron transport system, located within the plasmalemma, which can be linked to other membrane transport phenomena.

MATERIALS AND METHODS

Fusicoccin was prepared according to a published procedure (2); licorine was a kind gift of Prof. G. Randazzo (Istituto di Chimica Organica e Biologica, Università di Napoli); NADH, NADPH, Cyt *c*, and BSA were purchased from E. Merck, Darmstadt.

Maize roots were obtained from seeds of *Zea mays* L., var XL 342 (Italian DeKalb, Mestre, Italy), germinated on vermiculite wetted with H_2O for 4 d in the dark at 27°C. From the root, a segment about 5 mm long containing the tip was discarded, and the next 4 cm of root were excised in two segments 2 cm long weighing about 20 mg each, that were washed for 1 h in distilled H_2O and used immediately afterwards.

For determination of ferricyanide reduction, the root segments (100 mg/ml) were transferred in 1 mM Tris-HCl (pH 7.2) containing 0.5 mM $CaCl_2$ and, as required, variable quantities of salt and $K_3Fe(CN)_6$ and, where needed, 10 μM fusicoccin; the reaction was followed by the decrease in A_{420} (ϵ = 1.0 $mM^{-1}\ cm^{-1}$).

Anaerobiosis was maintained by bubbling O_2-free N_2 through the solutions, which had been previously degassed with a vacuum pump for 15 min.

The pH of external medium was measured upon addition of the root segments and again every 30 min and at the end of each experiment net H^+ fluxes were calculated by back titration with 10 mM NaOH from the final to the initial pH.

For *in vitro* experiments, maize roots were homogenized in a Waring Blendor for 30 s in 10 mM citrate buffer (pH 5.5) containing 5 mM $MgSO_4$ and 250 mM sucrose. The homogenized tissue was then partially purified by a series of centrifugations at different speeds; two fractions were thereby obtained, one named 'mitochondrial fraction,' which sedimented between 1,085*g* and 4,340*g*, the other named 'microsomal fraction,' which sedimented between 9,750*g* and 100,000*g*. The whole operation was conducted at 4°C.

NAD(P)H-ferricyanide or Cyt *c* reductase activities were measured according to Moreau *et al.* (15); Cyt oxidase activities according to Yonetani (16). Proteins were determined with a modified Lowry procedure (10).

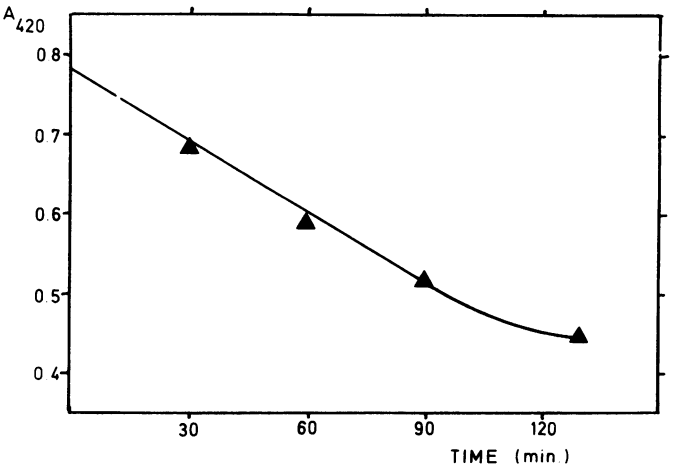

FIG. 1. Ferricyanide reduction by maize root segments. Three hundred mg of root segments were incubated in 3 ml of 1 mM Tris-HCl (pH 7) containing 0.5 mM $CaCl_2$ and 50 mM KCl. Temperature was kept to 27°C; reaction was started with 0.8 mM $K_3Fe(CN)_6$.

Table I. *Effect of Anaerobiosis on Ferricyanide Reduction and Fusicoccin-Induced Proton Extrusion*

Two hundred mg of root segments were incubated in 2 ml of 1 mM Tris-HCl (pH 6.5) containing 0.5 mM $CaCl_2$ and 50 mM KCl. FC and ferricyanide were added after 30 min of preincubation under air or N_2. All measurments were performed after 2 h of treatment. The figures are the means of four replications.

	Aerobiosis		Anaerobiosis	
	Proton extrusion	Ferricyanide reduction	Proton extrusion	Ferricyanide reduction
	μmol/h·g fresh wt			
Control	0.13		0.00	
$K_3Fe(CN)_6$, 1 mM	0.49	1.20	0.03	1.00
FC, 10 μM	2.53		0.08	
$K_3Fe(CN)_6$ + FC	3.11	1.45	0.30	1.17

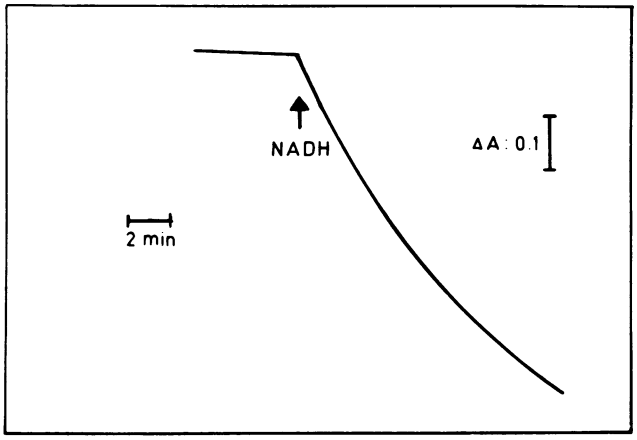

FIG. 2. Kinetics of NADH-ferricyanide reduction by the microsomal fraction from maize roots. NADH and ferricyanide were used at 1 mM concentration in 1 mM Tris-HCl (pH 7) containing 0.5 mM $CaCl_2$, 50 mM KCl, 300 mM mannitol, and 50 μg/ml of protein. Reaction was started by adding NADH; the molar rate in ferricyanide reduction *versus* NADH oxidation was found to be about 0.5. The experiments were repeated four times in triplicate. The values represent a typical experiment.

RESULTS AND DISCUSSION

Maize root segments reduce ferricyanide utilizing endogenous substrate with constant rate for 90 min (Fig. 1). Roots grown for the last 24 h in presence of 20 μM licorine, which inhibits the biosynthesis of ascorbate (1), showed the same behavior as reference sample. This rules out the hypothesis that ascorbate might have a relevant role in the redox process.

The lack of inhibition by 10 μM superoxide dismutase indicates that the electron donor is not a superoxide ion secreted by the cells since neither boiled roots nor the external medium after 3 h incubation with root segments have been shown to reduce ferricyanide, it follows that reducing agents released by the roots cannot account for the observed ferricyanide reduction.

Removal of the root segments and oxidation by H_2O_2 of the ferricyanide in the medium shows that none of the original oxidizing agent has been accumulated in the tissue. Anaerobiosis causes a decrease in the reduction rate of about 20%, which can be attributed to the lack of reducing agents due to the anaerobic condition itself.

The pH of the medium has little effect on ferricyanide reduction rate in the range from 5.5 to 8 in Tris-Mes buffer.

Ferricyanide reduction by root segments is favored in a medium of high ionic strength; stimulation by salts is relatively nonspecific, and can be accounted for as a screening effect against the negative surface charge of the cell, which would tend to repel ferricyanide ions (3). Kinetics of ferricyanide reduction show for both salt-stimulated and basal reaction the same V_{max} (3.1 μmol reduced substrate g^{-1} fresh weight h^{-1}) and K_m values of 1.4 mM with 5 mM KCl and 0.7 mM with 50 mM KCl concentration (Table I). This also supports the idea of an easier access of ferricyanide to the membrane surface owing to an increase in ionic strength.

A transmembrane oxidoreductase could involve the movement of protons through the membrane, if the final electron accepter, such as ferricyanide, does not become protonated when it is reduced. As shown in Table I, ferricyanide reduction is associated with external medium acidification. Fusicoccin, which actively stimulates proton extrusion, increases ferricyanide reduction rate by a mean 20%; on the other hand, in anaerobic conditions, ferricyanide has been shown to restore partially fusicoccin-induced proton extrusion. These data support the hypothesis that ferricyanide reduction is functionally related to proton extrusion.

As reported for lutoid membranes (15), a NADH-ferricyanide oxidoreductase activity is present in microsomes isolated from maize roots. Figure 2 shows the kinetics of ferricyanide reduction by these membranes. No reduction occurs unless NADH or microsomes are added. Initial velocities are proportional to the amount of membranes used in the assays, and the enzyme activity, which was found to be insensitive to cyanide, could not use NADPH as an electron donor or react with O_2. The reaction rate increases in a medium of high ionic strength, with maximal stimulation by Na^+ and K^+ at 0.1 M concentration. The reaction was also found to be pH-dependent, with an optimum between pH 7.5 and 8.5.

The microsomal fraction suffers little contamination by mitochondria as evidenced by the very low level of Cyt-oxidase and succinic dehydrogenase activity. On the other hand, the specific activity of NADH-ferricyanide oxidoreductase is lower in mitochondrial then in microsomal fraction. Upon heating to 50°C and 60°C, the NADH-ferricyanide activity was found to decrease by 50% in 7 and 3 min, respectively; it seems that inactivation is faster in the first 5 min, and proceeds thereafter at a slower rate.

Craig and Crane (4, 5) have recently described a transplasmalemma transport system in cultivated carrot cells, which reduced external and nonpermeant ferricyanide; in the meanwhile, Lin (11, 12) was able to demonstrate the existence of an electron transport system associated with plasmalemma of corn root cells that oxidizes external NADH.

The present study further supports this evidence. In addition, ferricyanide reduction, which is stimulated by fusicoccin, is accompanied by proton transport as in the system present in yeast (6) and, what is more, can partially restore anaerobic fusicoccin-driven proton extrusion.

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