K^+ - Dependent Uncoupling of Photophosphorylation by Nigericin*

by

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The proposed mechanisms of uncoupling in mitochondria and chloroplasts assume that either the uncoupler acts by catalyzing the hydrolysis of a high-energy intermediate (chemical theory; see Slater, 1967) or that the uncoupler discharges the proton potential across the membrane (chemi-osmotic theory; Mitchell, 1966). Uncoupling of oxidative phosphorylation by gramicidin or valinomycin has been explained according to the chemi-osmotic theory, as a discharge of the membrane potential by virtue of the enhanced alkali metal cation permeability induced by these antibotics. However, no example of uncoupling dependent upon the movement of alkali metal cations is known for photophosphorylation by chloroplasts.

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Graven et al. (1966), showed that nigericin causes the loss of alkali metal cations accumulated in mitochondria. Nigericin acts in mitochondria as an inhibitor of the oxidation of many NAD-linked substrates and of ATP $\rightleftharpoons$ Pi exchange; however, it does not act as an uncoupler of oxidative phosphorylation.

The data reported herein show that nigericin, in the presence of K^+ ions, is one of the most potent of the known uncouplers of photophosphorylation. It also inhibits the light-triggered ATPase, provided that K^+ ions are present. This pattern of inhibition suggests that nigericin may act in chloroplasts at the level of the ion translocating mechanism in the membrane.

Methods

Chloroplasts were isolated from fresh market spinach leaves by standard procedures, except that the homogenization medium contained 0.4 M sucrose and 0.01 M Tris-Cl, pH 7.8. ATP formation and ferricyanide reduction were assayed as described elsewhere (Shavit and Avron, 1967). ATP hydrolysis was assayed by measuring the ^{32}Pi released from AT^{32}P as described (Carmeli and Avron, 1966). Nigericin, a gift from Dr. R. L. Harned, Commercial Solvents Corporation, was kindly provided by Dr. D. Keister of this laboratory.

Results and Discussion

The effect of nigericin on electron transport and coupled phosphorylation with ferricyanide as electron acceptor, in a medium containing KCl, is shown in Fig. 1. It is clear that under these conditions nigericin is a powerful uncoupler of photophosphorylation; 50 percent inhibition of ATP formation was attained at 2×10^{-8} M. Furthermore,

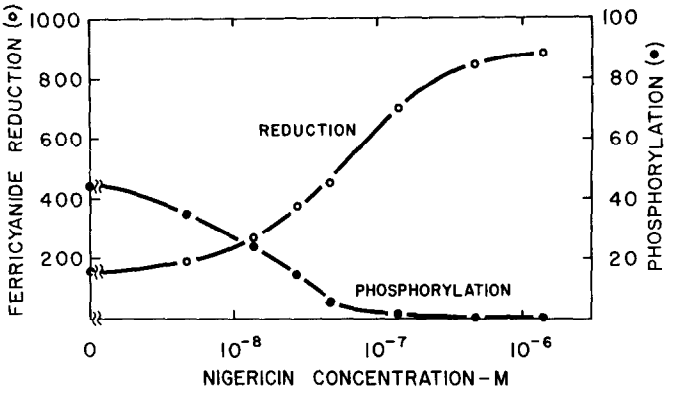

Figure 1: The effect of nigericin on ferricyanide reduction and coupled ATP formation.

Reaction mixture contained in a total volume of 3 ml at pH 7.8; 2 mM Tris-Cl, 2 mM $MgCl_2$, 0.33 mM ADP-Tris, 0.6 mM Pi-Tris, 0.25 mM $K_3Fe(CN)_6$, 50 mM KCl and 50-60 μg of chlorophyll. Temperature, 22°; light intensity, 80,000 lux for 30 sec. Ferricyanide reduction and ATP formation are given in μmoles per mg chlorophyll per hour.

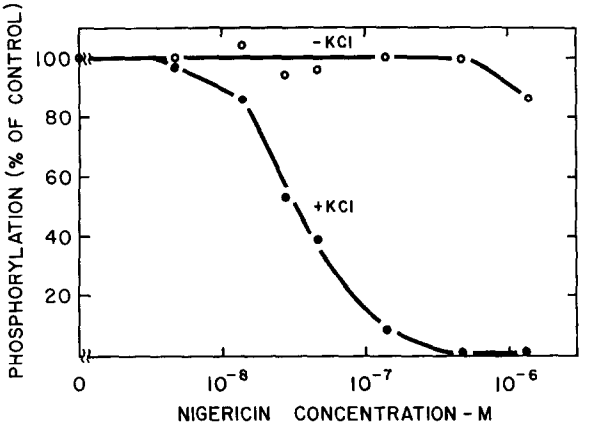

Figure 2: The effect of nigericin on ATP formation with PMS in the presence and absence of KCl.

Reaction mixtures as described in Fig. 1, except for the presence of 0.03 mM PMS and where indicated, 50 mM KCl. Control rates were 290 and 450 μmoles ATP per mg chlorophyll per hour, in the absence and presence of KCl, respectively.

a 5 to 6-fold stimulation of the rate of electron transport was obtained concurrent with inhibition of ATP synthesis. The experiment described in Fig. 1 was performed with a phosphate acceptor system present. Although not shown, a similar degree of stimulation of electron transport by nigericin was observed in the absence of a phosphate acceptor system. In each case, maximal stimulation was observed in the presence of 50-100 mM KCl; no stimulation was observed in the absence of KCl.

Nigericin inhibits ATP formation both with ferricyanide and PMS, and the inhibitory pattern is comparable in each case. As illustrated in Fig. 2, nigericin inhibits ATP formation with PMS provided that K^+ ions are present. In their absence, and with concentrations of nigericin of up to 10^{-6} M, no significant inhibition was observed. The effect of nigericin on ATP formation in the presence of various alkali metal cations is given in Table I. Since Na^+ and Li^+ ions do not replace K^+ efficiently, it seems that K^+ ions are specifically required for the inhibition by nigericin to occur.

Table I

The effect of nigericin in the presence of various alkali metal cations

Cation Added	Nigericin concentration		
	None	3×10^{-8} M	5×10^{-8} M
	μmoles ATP formed per mg chlorophyll per hour		
None	268	268	315
K^+, 50 mM	354	247	172
Na^+, 50 mM	336	313	310
Li^+, 50 mM	326	326	308

Reaction mixtures and assay conditions as in Fig. 2

Comparison of uncoupling of photophosphorylation and oxidative phosphorylation indicates that a systematic difference exists in the effect of various compounds on chloroplasts and mitochondria (Mitchell, 1966). Valinomycin, which is known to enhance permeability of membranes to specific cations and to uncouple oxidative phosphorylation, has very little effect on photophosphorylation (Avron and Shavit, 1965). Preliminary experiments indicate that nigericin inhibits the light-dependent H^+ ion uptake and the formation of ATP, both in the two stage phosphorylation assay and in the acid-base assay provided that K^+ ions are present in the medium. Nigericin also appears to induce both a K^+ for H^+ exchange in chloroplasts in the dark and a light-induced swelling of chloroplasts in the presence of K^+ ions (Shavit et al. 1967).

It is suggested therefore that nigericin represents the first example of uncoupling of photophosphorylation by an agent which appears to affect primarily the permeability of chloroplasts to specific alkali metal cations.

The light dependent efflux of K^+ ions (Dilley and Vernon, 1965) and uptake of H^+ ions (Jagendorf and Neumann, 1965) observed in chloroplasts has been suggested to result in the formation of a high energy intermediate or state which energizes Pi and ADP to form ATP. The light-triggered ATP hydrolysis in chloroplasts (Petrack et al., 1965) is a reaction which occurs in the dark after light induction. We therefore studied the effect of nigericin on this hydrolytic activity in the dark. As shown in Table II, in the presence of K^+ ions the ATPase activity was inhibited by nigericin. In their absence, stimulation rather than inhibition was observed. Furthermore, the alkali metal specificity for inhibition by nigericin of ATPase was the same as that reported for phosphorylation (Table I).

Table II

Effect of nigericin on light-triggered ATPase

Conditions	Expt: I percent of control	II percent of control
Control	100	100
+ Nigericin, 4×10^{-7} M	151	---
+ KCl, 50 mM	---	105
+ KCl, 50 mM and nigericin, 5×10^{-7} M	---	10

Reaction mixtures contained in a total volume of 3 ml the following components in μmoles: Tris-Cl pH 7.8, 10; $MgCl_2$, 5; AT^{32}P-Tris, 2; Dithiothreitol-Tris pH 7.8, 75; PMS, 0.1; and chloroplasts containing 60 μg chlorophyll. ATP, KCl and nigericin were added in the dark after a 2 minute illumination period at 80,000 lux. At the end of 5 minutes, the reaction was terminated by the addition of TCA to a final concentration of 3 percent ($^{v}/v$) and the amount of ^{32}Pi released was measured. Control rates: 137 and 118 μmoles Pi released per mg chlorophyll per hour, for Expt. I and II, respectively.

As indicated above, the observed loss of K^+ ions induced by nigericin in the dark (Shavit et al., 1967) cannot be the cause for the uncoupling of photophosphorylation, since the latter was observed only in the presence of externally added K^+ ions. Although the loss of K^+ cannot explain the inhibition pattern, it nevertheless indicates an increased permeability of chloroplasts to K^+ ions. In terms of the chemiosmotic theory, nigericin may affect the exchange diffusion system, enhance K^+ influx, and thus dissipate the proton gradient. It still remains to be shown, however, that in a medium containing KCl, nigericin induces the accumulation of K^+ inside the chloroplasts. The stimulation of ATP hydrolysis by nigericin, in the absence of K^+ ions, may occur by facilitating K^+ efflux and consequent internal accumulation of H^+ ions.

It is suggested that nigericin acts at the level of the ion transport mechanism in the membrane, in a manner analogous to the action of the transport-inducing antibiotics in mitochondria. The effect of nigericin on proton uptake, ATP formation reactions, movements of alkali metal cations and volume changes of chloroplasts will be presented in a forthcoming report (Shavit et al., 1967).

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