EFFECT OF NIGERICIN UPON LIGHT-DEPENDENT MONOVALENT CATION TRANSPORT IN CHLOROPLASTS

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The formation of proton gradients in chloroplasts by light (Neumann and Jagendorf, 1964), the light-triggered ATP hydrolysis (Packer, 1963), or by acid-base transitions in the presence of weak acid anions (Uribe and Jagendorf, 1967), occupies a central position in the current efforts to comprehend energy transduction associated with ion transport, conformational changes, and photophosphorylation in chloroplasts (Packer and Crofts, 1967). Evidence upon which this conclusion is based rests upon observations that H+ gradients enhance the rate and extent to which energy-dependent processes occur. Conversely, factors which inhibit or dissipate proton gradients, i.e. uncouplers, inhibit energy-dependent processes. Not only is it presently unclear as to how proton gradients arise by light or ATP hydrolysis, but clarification is also required as to whether these gradients constitute a primary energetic event.

In several recent investigations, like those of Plengvidhya and Burris (1965) and Shavit and San Pietro (1967), the effect of various antibiotic reagents upon electron and energy transfer reactions in chloroplasts has been examined with the view to uncovering reagents which exert a specific action upon these reactions. Such studies are relevant to distinguishing between alternative mechanisms, especially chemical or chemiosmotic (Mitchell, 1966), as a basis for the energetic state. It has now been discovered that the antibiotic substance, nigericin, which inhibits ion transport in mitochondria (Graven, Estrada-O., and Lardy, 1967), exerts a highly specific monovalent cation-dependent inhibition of light-induced proton transport in chloroplasts.

The action of certain antibiotics on light-induced light scattering increments (or transmission decreases) in isolated spinach chloroplasts is shown in Table I. It is evident that of the six substances examined, only nigericin exerted an inhibitory effect. Nigericin did not appreciably inhibit conformational changes in Tris-acetate medium whereas in Na- or

K-acetate complete inhibition was observed. This inhibition was not manifested when monovalent alkaki-metal cations were absent from the medium.

TABLE I

Action of Antibiotic Substances on Light-Induced Changes of Chloroplast Conformation

Basic conditions (V_f = 3 ml): Na-acetate, K-acetate at pH 8, except Tris-acetate at pH 7.5, acetate at 100 mM; PMS, 10 μM; chlorophyll, 10 μg/ml. Antibiotics tested at 1 μM levels by adding 60 μl of 5 x 10^{-4} M ethanol solution. Percent decreases in transmission (T) or increases in 90° light scattering (LS) at 546 mμ were recorded simultaneously following illumination of spinach chloroplasts with red actinic light as previously described (Packer, 1963, 1967).

	Light-Induced Change (percent)					
	Tris-acetate		Na-acetate		K-acetate	
	+LS	-T	+LS	-T	+LS	-T
Control	87	20	100	28	78	24
Streptonigrin	82	21	80	23	84	23
Ossamycin	86	22	82	25	88	24
Peliomycin	90	23	84	23	92	23
Venturocidin	90	23	86	22	90	23
Nigericin	84	21	0	0	0	0
Nonactin	80	18	84	26	66	24

This observation led to further investigations of the nigericin action. Electron flow was found to be not inhibited by nigericin, even in media containing monovalent metal cations, as judged by its inability to quench chlorophyll fluorescence (615 mμ excitation/715 mμ measurement) or inhibit the ferricyanide Hill reaction (ferricyanide reduction and O_2 evolution). However, photophosphorylation and the light-triggered hydrolysis of ATP were found to be inhibited as reported by Shavit and San Pietro (1967). Estrada-O., Graven and Lardy (1967) observed that nigericin elicits a K-specific hydrolysis of ATP in mitochondria. Hence, attention to the effect of this reagent upon light-dependent cation transport was examined. Nigericin was found to inhibit light-induced H+ uptake (0.32 μ eq/mg chlorophyll in Fig. 1) when the suspending medium contains monovalent metal cations. The effective inhibitory concentration is in the range of 10^{-7} to 10^{-8} M with the actual inhibitory concentration depending upon the nature of the cations present in the medium. Figure 1 (top trace) illustrates that release of proton uptake by nigericin is rapid; 0.1 μM causes approximately 50 percent inhibition of proton uptake and 0.2 μM nigericin is sufficient to abolish proton gradients.

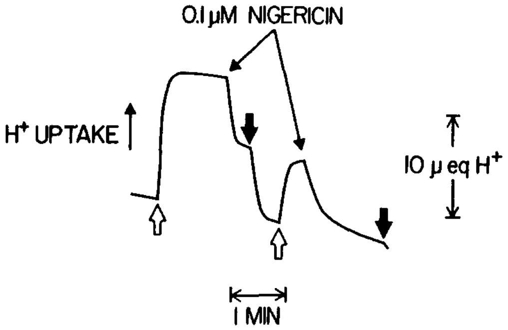

Figure 1. Inhibition of light-induced H^+ uptake in chloroplasts by nigericin. The reaction mixture (7.5 ml) contained 114 mM NaCl, 0.75 mM Na-phosphate, 0.4 mM Tris-Cl, 4 μM phenazine methosulfate (PMS), and chloroplasts (40 μg chlorophyll/ml) at pH 6. Spinach chloroplasts were isolated in 350 mM NaCl-10 mM Tris-Cl at pH 8. The test system was illuminated with tungsten light at 4000 foot-candles. Open arrows pointing upward indicate the time when illumination was made; dark arrows pointing downward signify cessation of illumination. H^+ changes were recorded with a Radiometer M26 Meter employing a combination type pH electrode.

In the absence of alkali metal ions as in choline chloride medium, concentrations of nigericin even as high as 1 μM exert no inhibitory effect upon proton transport. Figure 2 demonstrates uptake (corresponding to 0.33 μ eq/mg chlorophyll) and release of protons by chloroplasts in a choline chloride medium during a cycle of illumination and darkness in the presence of nigericin. The addition of 8 μM KCl in the dark causes no effect upon the proton trace and a subsequent cycle of illumination and darkness shows that proton transport is hardly affected. However, when the concentration of KCl is raised to 88 μM by the addition of 80 μM KCl, accumulation of protons by illuminated chloroplasts was prevented. The specificity of this inhibitory effect of alkali metal cations on proton uptake has also been investigated. It was observed that proton uptake by nigericin-treated illuminated chloroplasts is not inhibited by bivalent cations like Ca^{++} and Mg^{++}, whereas other alkali monovalent cations like Na^+ are effective, Complete inhibition of proton uptake was brought about by 1 mM Na^+, thus it is about 10-fold less effective than K^+ on the basis of concentration.

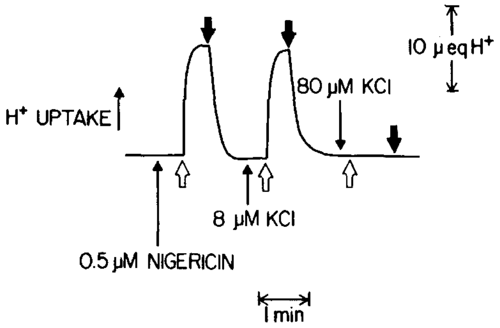

Figure 2. Monovalent alkali cation dependence of the nigericin inhibition of light-induced H^+ uptake in chloroplasts. The reaction medium (7.5 ml) contained: 100 mM choline chloride, 0.9 mM Tris-Cl, 4 μM PMS, and chloroplasts (40 μg chlorophyll/ml) at pH 8.0. The chloroplasts were isolated in a 100 mM choline chloride-10 mM Tris-Cl medium at pH 8.0. Other conditions as in Fig. 1.

The question now is, by what mechanism does nigericin interfere with proton transfer? Crofts, Deamer and Packer (1967) have demonstrated a light-dependent potassium uptake which occurs in chloroplasts which is ordinarily quite small in relation to proton uptake. Also Nobel and Packer (1965) have described a light-induced Na+ uptake by chloroplasts. As it seemed possible that nigericin might specifically facilitate the uptake of monovalent alkali metal cations, experiments were designed to test this idea directly by means of ion sensitive electroses. Figure 3 demonstrates the results of typical experiments performed in choline chloride medium. Illumination of chloroplasts (as in upper trace) causes a very small uptake of some unidentified cations, which is reversible in darkness as previously reported (Crofts, et.al., 1967). Addition of nigericin causes no change in the cation trace in the dark, but upon illumination a significantly larger uptake of cations is observed. Note that the calibration for H+ is negative while the electrode responds quite strongly to much lower concentrations of K+. The lower trace in Fig. 3 shows that in the presence of 10 μM K+, light-induced cation uptake is not observed, partially because the electrode response is to K+. However, note that 0.5 μM nigericin causes a subsequent light-induced K+ uptake. Since 10 μM KCl does not appreciably inhibit H+ uptake (cf. Fig. 2) the uptake of K+ under these conditions is small (2.5 m μ eq K+).

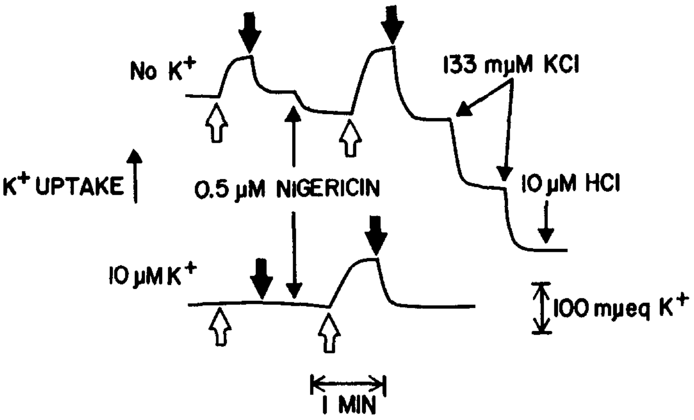

Figure 3. Nigericin induced light-dependent K$^+$ uptake by chloroplasts. Chloroplasts were isolated and tested (at 10 μg chlorophyll/ml) in a medium containing 100 mM choline chloride, 10 mM Tris-Cl at pH 8.0. The medium was supplemented with 4 μM PMS and additions as indicated. Other conditions as in Fig. 1, except that the following electrodes were employed: cation sensitive (type GKN-33, Electronic Instruments Ltd., Richmond, Surrey, England); reference electrode, Ag-AgCl fiber junction (type 39168 A4 Beckman Instrument Co., Fullerton, California). These electrodes were sensitive to K$^+$ and insensitive to changes in H$^+$ under the conditions of these experiments and shown by the calibrations of K$^+$ and H$^+$. The Na+ response by the electrodes was 3.3 fold less sensitive than K$^+$.

Similar experiments performed with concentrations of K+ and Na+ inhibitory to H+ uptake have shown that nigericin can induce extensive cation uptake. Examples of nigericin-induced uptake in illuminated chloroplasts are: a) K+ uptake of 0.055 μ eq/mg in 92 μM KCl; b) Na+ uptake of 2.25 μ eq/mg in 1.33 mM NaCl. Evidently the extent of nigericin induced Na+ uptake much exceeds that for H+ uptake in illuminated chloroplasts. Although on a concentration basis K+ more readily inhibits H+ uptake, we have thus far observed that nigericin induces more extensive uptake of Na+ than K+.

This investigation has established that nigericin specifically facilitates the uptake of monovalent alkali metal cations in illuminated chloroplasts at the expense of H+ uptake. Disappearance of the light-dependent proton gradient is accompanied by loss of light-induced conformational changes, inhibition of photophosphorylation and the light-triggered hydrolysis of ATP. These results strengthen our earlier suggestion (Packer and Crofts, 1967) that ion transport in illuminated chloroplasts competes for the energy required for photophosphorylation by dissipating proton gradients. Under normal circumstances the principle monovalent cation accumulated by chloroplasts is H+ with only a small proportion of the ion uptake due to other monovalent cations (Crofts, et.al., 1967). When, however, a reagent facilitating potassium transport, is incubated with chloroplasts, illumination favors uptake of Na+ and K+ at the expense of H+. Hence, these results focus attention upon the coupling of electron to energy transfer as involving a mechanism not inextricably linked to H+ transport.

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