*INHIBITION BY NIGERICIN OF THE LIGHT-INDUCED PH CHANGE IN RHODOSPIRILLUM RUBRUM CHROMATOPHORES**

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An important aspect of energy conservation in mitochondria, chloroplasts, and chromatophores is the temporal and sequential interrelationships between electron transport, proton movements (and the resultant proton gradient), high-energy intermediates or states, and the ultimate formation of adenosine 5′-triphosphate (ATP).[1-4] At present, two quite dissimilar alternative postulations are under consideration. The first assumes that proton movements are a prerequisite for the establishment of a high-energy state;[1, 4] the other views proton movements as a consequence of the formation of a high-energy state.[2, 3]

Within recent years, a number of antibiotics have been shown to induce ion transport in mitochondria[5, 6] and in chloroplasts.[7-9] These antibiotics are useful to investigate the interrelationship between the proton and alkali metal-cation movements and the mechanism for energy conservation.

Data presented in this communication demonstrate that low concentrations of nigericin, an antibiotic which inhibits proton uptake and ATP formation in chloroplasts,[7, 8] strongly inhibits the light-dependent proton movements in chromatophores without affecting the rate of ATP formation. These results suggest that proton uptake in chromatophores represents a side reaction not on the pathway of ATP formation.

Methods.—Rhodospirillum rubrum, S1, was grown and chromatophores prepared as described previously.[10] For measurement of light-induced pH changes, the crude chromatophore preparation was washed twice with 0.35 *M* NaCl instead of 0.1 *M* Tris-Cl, pH 8.0, and 0.3 *M* sucrose. ATP^{32} formation was assayed as described earlier.[11] Bacteriochlorophyll was determined from the *in vivo* absorption at 880 nm.[12] pH changes in an 8-ml reaction mixture were measured with a Leeds and Northrup model 7405 pH meter with a glass electrode and a Ag/AgCl reference electrode. Red-light illumination was obtained using a Unitron model LKR illuminator with a Corning 2304 red filter, and a CS-69 infrared-absorbing filter. Light intensity was 8×10^5 ergs per square cm per sec. The gas phase was air and the temperature 22°.

Results.—Typical traces of the pH change observed upon illumination of suspensions of isolated chromatophores are shown in Figure 1. The steady-state level (extent) of the light-induced pH rise or proton uptake attained in a medium containing 0.1 *M* KCl (Fig. 1*A*) was rapidly reduced by addition of 4.7×10^{-8} *M* nigericin in the light. The attenuated extent corresponds to a decrease of about 70 per cent from the original extent. Complete reversal of the pH rise occurred in the dark. A second period of illumination again produced a pH rise but only to the inhibited steady-state level. Nigericin inhibited both the apparent rate of formation and the extent of the pH rise.

Von Stedingk and Baltscheffsky[13] have shown that valinomycin stimulates the rate of the light-induced pH rise in chromatophores in a medium containing KCl. This stimulation by valinomycin of the light-induced proton uptake was

confirmed. Nigericin also inhibited the light-induced proton uptake stimulated by valinomycin (unpublished results).

The effect of nigericin in mitochondria[6] and chloroplasts[7] is selective for K^+. Comparison of the results presented in Figure 1 shows that nigericin was more

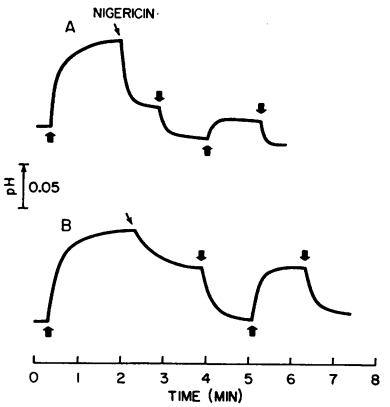

FIG. 1.—Effect of nigericin on the light-induced proton uptake of *R. rubrum* chromatophores. Reaction mixtures contained 0.02 mM succinate, 0.1 *M* KCl (*A*) or NaCl (*B*), and 14 μg bacteriochlorophyll per ml. Initial pH was 6.3. Nigericin, 4.7×10^{-8} *M*, was added where indicated (↘); (↑, ↓) indicate light on and off, respectively. Forty-two mμmoles of H^+ corresponded to a shift of 0.1 pH unit in a 6-ml reaction mixture.

effective in the presence of K^+ than Na^+ in *R. rubrum* chromatophores. In a medium containing 0.1 *M* NaCl (Fig. 1*B*), the addition of 4.7×10^{-8} *M* nigericin in the light reduced the extent of the pH rise by only 40 per cent and at a much slower rate than in the presence of KCl. An additional period of illumination again brought about a rise in pH but only to the inhibited steady-state level. The inhibition of the apparent rate of formation was much less pronounced than in the case of KCl (Fig. 1*A*).

Despite the inhibition of the pH rise in chromatophores, the rate of ATP formation was not affected by low concentrations of nigericin. This is in contrast to the effect on photophosphorylation in chloroplasts, where nigericin uncouples electron transport from photophosphorylation.[7, 8] The differential effect of nigericin on the light-induced pH rise and on the rate of ATP formation as a function of nigericin concentration is given in Figure 2. Fifty per cent inhibition of the extent and of the apparent rate of formation of the pH change were obtained at 4×10^{-9} *M* and 3×10^{-8} *M* nigericin, respectively. Much higher concentrations of nigericin were required to inhibit ATP formation. At 10^{-7} *M* nigericin, the extent of the pH rise was 86 per cent inhibited, whereas photophosphorylation was unaffected. This differential inhibition of the light-induced pH change, observed in the absence or presence of succinate, was not dependent upon the rate of ATP formation. That is, the same result was obtained under conditions which support either low or high rates of ATP formation.

It was necessary to ascertain that this differential inhibition of the light-induced pH change, but not of ATP formation, was not the result of the different experimental conditions required for each assay. We therefore compared the effect of nigericin on both the rate of ATP formation and the pH rise in the presence of AsO_4 (instead of P_i) at pH 7.4. As shown in Figure 3*A*, the curve obtained for the change in pH in the presence of P_i was biphasic. The initial

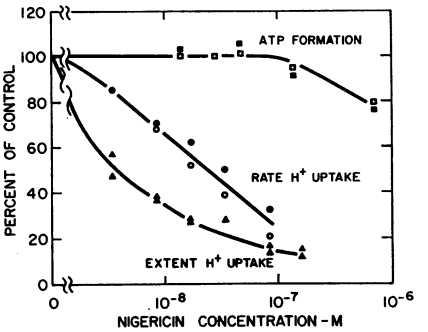

FIG. 2.—Effect of nigericin on the light-induced proton uptake and photophosphorylation.

Reaction mixtures for measurements of the light-induced pH change contained 0.1 *M* KCl and chromatophores, washed twice with NaCl, corresponding to 11 μg bacteriochlorophyll (BChl) per ml.

Solid symbols: reactions performed in the presence of 0.02 mM succinate; *open symbols:* in the absence of succinate. Initial pH was 6.3. The control values were 84 μmoles H^+/mg BChl/hr for the initial rate of proton uptake and 0.27 μmole H^+/mg BChl for the extent of the reaction.

The reaction mixtures for the photophosphorylation assay contained 3.3 mM P_i^{32} (1×10^6 cpm); 3.3 mM $MgCl_2$; 1.67 mM ADP; 100 mM KCl; 10 mM tris-Cl, pH 8.0, and chromatophores, washed twice with sucrose-tris, corresponding to 10 μg BChl per ml. Solid and open symbols are as indicated above. Time of illumination was 3 min. The reaction was stopped by addition of 0.3 ml; 50% TCA and ATP^{32} was assayed as described. Control activities were 153 and 24 μmoles ATP formed/mg BChl/hr, with and without succinate, respectively.

rapid phase can be attributed to the light-induced pH rise and the slower phase to the formation of ATP.[14, 15] The addition of nigericin at 1×10^{-7} *M* inhibited the initial rapid change in pH without affecting the slower phase (not shown). With 1.7×10^{-6} *M* nigericin, the rapid phase was completely abolished whereas the pH change due to ATP formation was inhibited only 30 per cent (Fig. 3*A*).

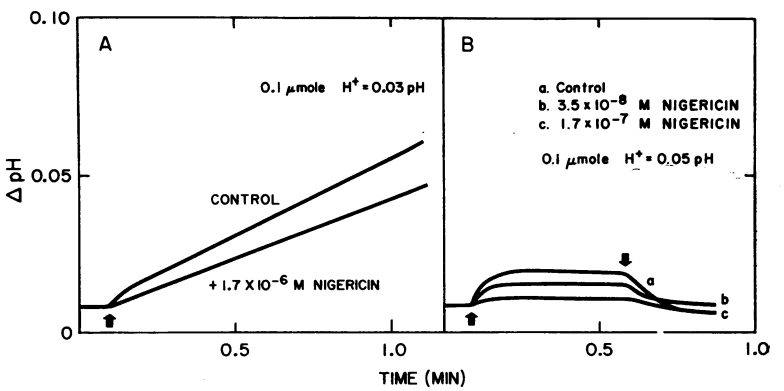

FIG. 3.—Inhibition by nigericin of the light-induced pH change in the presence of Mg^{++}, ADP, and P_i or arsenate. Reaction mixtures contained 100 mM KCl; 0.2 mM ADP; 0.5 mM $MgCl_2$; 0.02 mM succinate; chromatophores, washed twice with NaCl, corresponding to 23 μg BChl/ml; and 0.2 mM P_i (*A*) or 0.2 mM arsenate (*B*). Initial pH was 7.4.

When P_i is replaced by AsO_4, there is no pH change which is attributable to the formation of ATP. Under these conditions, the pH change observed is probably similar to that noted in the absence of ADP, AsO_4, and Mg^{++}. Hence, the effect of nigericin on this pH rise should be similar to that observed in the

absence of a phosphate acceptor system. That this is the case is shown in Figure 3*B* in that low concentrations of nigericin strongly inhibited this pH rise. It is worth noting that the initial rapid pH change observed in the absence of nigericin (Fig. 3*A*) is similar to that seen in the presence of AsO_4, when corrections are made for different buffer capacities.

The formation of ATP^{32} was determined to establish further the effect of nigericin under conditions similar to those used in the experiments of Figure 3*A*. Using low concentrations of P_i and short periods of illumination, it was found, as shown in Figure 4, that 1.5×10^{-8} *M* nigericin did not inhibit ATP formation and 1.5×10^{-7} *M* nigericin was only slightly inhibitory (about 10%).

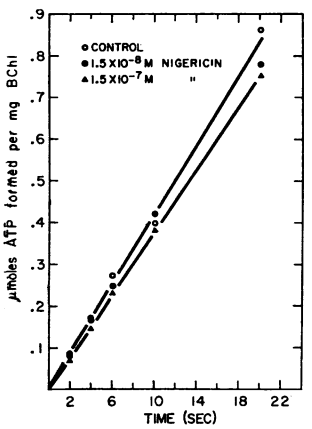

FIG. 4.—Effect of nigericin on initial rate of ATP formation. The reaction mixtures contained 0.2 mM $^{32}P_i$ (1×10^6 cpm): 0.5 mM $MgCl_2$; 0.8 mM ADP; 100 mM KCl; 10 mM tris-Cl, pH 8.0; 0.02 mM succinate and chromatophores, washed twice with sucrose-tris, corresponding to 19 μg BChl per ml. The control rate was 140 μmoles ATP formed/mg BChl/hr.

Nigericin has been reported to inhibit the transport of P_i in mitochondria.[16] A comparison of the experiments presented in Figures 2 and 4, which were performed using different concentrations of P_i, demonstrate that the lack of inhibition of ATP formation cannot be attributed to the high levels of P_i used in the experiments of Figure 2. It is also clear that nigericin had only a very slight effect on ATP formation even with short periods of illumination (Fig. 4).

The effect of nigericin on the energy-linked transhydrogenase present in *R. rubrum* chromatophores[10] is shown in Table 1. This reaction is postulated to utilize a high-energy intermediate of phosphorylation. The low levels of nigericin which inhibited the pH rise (Fig. 2) had no effect on the transhydrogenase reaction. This is in agreement with the lack of effect of nigericin on photophosphorylation. At higher concentrations of nigericin, the transhydrogenase activity was inhibited using either light or ATP as the source of energy. The inhibition at higher concentrations of nigericin, which was also observed for photophosphorylation, is possibly due to an additional effect of nigericin, similar to that observed in mitochondria.[17] Again, the effectiveness of nigericin was dependent on the presence of salt and was more specific for K^+ than for Na^+.

Nigericin inhibited the ATP-supported reaction somewhat more than the light-supported reaction. This corresponds with the effect of some known uncoupling agents on this energy-linked enzyme reaction.[10]

Discussion.—Nigericin inhibits proton uptake and uncouples electron transfer from ATP formation in chloroplasts.[7, 8] The collapse of the proton gradient established upon illumination is believed[8, 18] to ensue from the induction of an inward flow of K^+. Thus, nigericin in chloroplasts acts as a typical uncoupling agent which, according to the chemiosmotic hypothesis,[1] should short-circuit

TABLE 1. *Effect of nigericin on energy-linked transhydrogenase.*

Nigericin	Energy Source: Light −KCl	Light +KCl	ATP −KCl	ATP +KCl	ATP +NaCl
	(Per cent inhibition)				
1.4×10^{-8} *M*	0	2	0	7	3
1.4×10^{-7} *M*	0	7	0	22	17
4.7×10^{-7} *M*	2	9	0	24	22
1.4×10^{-6} *M*	4	18	0	35	25
4.7×10^{-6} *M*	5	49	0	79	27

The transhydrogenase was assayed as previously described.[10] The control rates of $NADP^+$ reduction were 42.6 and 24.8 μmoles/mg BChl/hr for light and ATP as energy sources, respectively. These rates were inhibited about 38% by the inclusion of 0.1 *M* KCl or NaCl.

the proton current and uncouple electron transfer from ATP formation. Lardy *et al.*[6] have suggested that nigericin-type antibiotics act in mitochondria by interfering with the availability of a high-energy intermediate to the mitochondrial ion pump. Pressman *et al.*,[19] on the other hand, believe that nigericin provides a bypass for cations through which gradients of K^+ and H^+ are discharged without affecting oxidative phosphorylation. Studies of the effect of nigericin in chloroplasts[8, 18] have not clearly disengaged the K^+/H^+ exchange from the energy conservation mechanism, since K^+ uptake and H^+ efflux induced by nigericin occur concomitant with uncoupling.

The effect of nigericin in *R. rubrum* chromatophores allows for an evaluation of the relationship between proton movements and ATP formation. It appears that interference by nigericin with the mechanism of ion translocation, resulting in the collapse of the proton gradient, has little effect on ATP formation in chromatophores. These results are relevant to the chemiosmotic hypothesis proposed by Mitchell.[1] In this postulation, proton movements are viewed as a primary process, compulsorily linked to electron transport, and a necessary prerequisite for ATP formation. One may deduce, therefore, that any impairment of proton movements should result in a corresponding effect on ATP formation.

We conclude, therefore, that there is no direct and necessary relationship between proton movements and ATP formation in *R. rubrum* chromatophores. A similar view was expressed by Chance *et al* [20] and von Stedingk and Baltscheffsky.[13] The former authors showed that the rate of proton movements in *R. rubrum* chromatophores is too slow to be compulsorily linked to light-activated electron transport.

Summary.—Low concentrations of nigericin, in the presence of K^+, inhibit the light-induced pH change and have no effect on the rate of ATP formation in *R. rubrum* chromatophores. It is concluded that proton uptake in chromatophores represents a side reaction not on the pathway of ATP formation.

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