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Inhibitor Studies on Light-Induced Phosphorylation in Extracts of *Rhodospirillum rubrum*

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Light-induced phosphorylation in extracts of the photosynthetic bacterium *Rhodospirillum rubrum* is strongly inhibited not only by 2-*n*-heptyl-4-hydroxyquinoline-N-oxide [1] and antimycin A[2] but also by gramicidin and oligomycin A in concentrations which are similar to those necessary to inhibit the reactions involved in oxidative phosphorylation in animal mitochondria. Amytal, azide and cyanide [3] have only a weak inhibitory effect. Dicumarol and especially 2,4-dinitrophenol, which both "uncouple" phosphorylation from electron transport in animal mitochondria, have a much less pronounced inhibitory effect on the light-induced phosphorylation.

The similarities between the two different electron transport phosphorylation * systems in response to various kinds of inhibitors indicate that the investigated mechanism for bacterial light-induced phosphorylation has properties similar to the mechanism for mitochondrial oxidative phosphorylation. This is true with respect not only to electron transport but also to the mechanism of coupling between electron transport and phosphate esterification and to the nature of a reaction involved in phosphate transfer. The differences show that reactions near the oxidizing and the reducing ends of the mitochondrial respiratory chain are not shared by the bacterial system for light-induced phosphorylation.

Studies with inhibitors and stimulators have provided information about the system for respiration and oxidative phosphorylation in animal mitochondria. Experimental evidence for the assumption that light-induced phosphorylation (LIP) in photosynthetic bacteria is dependent upon electron transport was first obtained with the electron transport inhibitor HOQNO [1] **. Current concepts about the electron transport system in LIP in the facultative

* The term "electron transport phosphorylation" is used to denote "phosphorylation linked to electron (or hydrogen) transport".

** Abbreviations: P, orthophosphate; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; HOQNO, 2-n-heptyl-4-hydroxyquinoline-N-oxide; DNP, 2,4-dinitrophenol; % P_{org}, percentage orthophosphate esterified; M, moles per liter.

phototroph *Rhodospirillum rubrum* are largely based on results obtained with electron transport inhibitors and stimulators [1-4]. Further results obtained with inhibitors of electron transport and phosphorylation are given and discussed in this paper.

MATERIALS AND METHODS

ATP, DPNH, PMS and antimycin A were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Gramicidin D was obtained from Mann Research Laboratories, New York, N.Y., U.S.A. HOQNO was obtained from Dr. J. W. Cornforth, National Institute for Medical Research, London, England, and oligomycin A from Dr. F. M. Strong, Department of Biochemistry, University of Wisconsin, Madison, Wisc., U.S.A. These gifts are gratefully acknowledged. Hexokinase was prepared and stored as in Ref.[5] Antimycin A, HOQNO, gramicidin and oligomycin A were dissolved in ethanol. The stock solutions were kept in a deep-freeze. In the reaction medium for LIP the ethanol content was kept below 1 % in order not to inhibit the LIP. The molecular weight of antimycin A was taken as 548 (*cf.* Ref.[6]).

Rhodospirillum rubrum, van Niel strain S1, was grown and harvested as has been described [4]. The method which was used for disruption of the bacteria, grinding with alumina, was first employed on bacteria by McIlwain [7]. It has been described in a previous paper [4], as well as the procedures employed for preparation of "chromatophores" and "chromatophore fragments" and for measuring yield and efficiency of LIP, and the medium used in the LIP experiments. The results reported here have been obtained with the "chromatophore fragments" fraction unless otherwise indicated. The two fractions were equally efficient in LIP but the "chromatophores" fraction showed a higher DPNH-oxidase activity than the "chromatophore fragments" fraction.

Aerobic conditions were used throughout. In all experiments 3.3×10^{-4} M * succinate was added in accordance with the finding of Frenkel [8,9] that a reducing agent in catalytic amounts is necessary to elicit full LIP activity in "purified" extracts of *R. rubrum*. Frenkel [9] has shown that the LIP process is inhibited by molecular oxygen in the absence, or at very low concentrations, of hydrogen donor. Under our conditions, with an added excess of succinate, anaerobiosis obtained with N_2 *via* a pyrogallol solution did not result in higher LIP values. In fact, a slight inhibition of the LIP as compared with aerobiosis was obtained (10 and 15 % in two separate experiments with different preparations).

RESULTS

Fig. 1 shows that the rate of LIP is a linear function of the concentration of the chromatophore extract. A time curve for LIP is given in Fig. 2.

Amytal gives only a weak inhibition of LIP, even at concentrations several times higher than those which strongly inhibit the oxidation of DPN-linked substrates in animal mitochondria [10] (Fig. 3).

Fig. 4 shows the inhibition curves for antimycin A and HOQNO, which have been shown [11] to inhibit electron transport in animal mitochondria between cytochrome b and cytochrome c_1. Fig. 5 shows that the inhibition obtained with antimycin A was by-passed by phenazine methosulfate [2,12], even at high concentrations of the inhibitor. However, with increasing concentrations of antimycin A the amount of phosphate esterified decreased.

As shown in Table 1, no marked inhibition was obtained with high concentrations of KCN and NaN_3. Mitochondrial electron transport is strongly inhibited by 5×10^{-4} M concentrations of these agents.

* Final concentrations are given throughout.

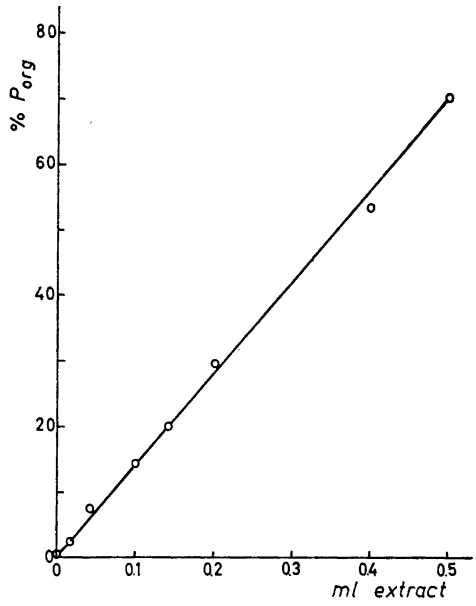

Fig. 1. LIP as a function of the amount of added "chromatophore fragments". The tubes contained 1.5 ml 0.2 M glycylglycine pH 7.4, 8 μmoles $K_2H^{32}PO_4$, 10 μmoles ATP, 1 μmole succinate, 30 μmoles $MgCl_2$, 60 μmoles glucose and an excess of yeast hexokinase. "OD_{800}" = 0.20 for a sample with 0.1 ml "chromatophore fragments" per 3.0 ml (reaction volume 3.0 ml). 20 min experiment at 30°C.

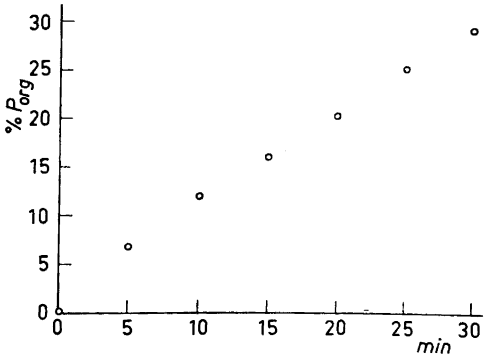

Fig. 2. Time curve for LIP. Experimental details as in Fig. 1. "OD_{800}" = 0.20.

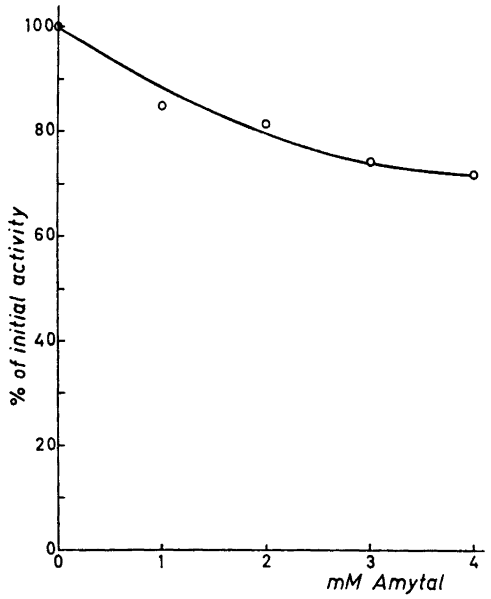

Fig. 3. Effect of amytal on LIP. Experimental details as in Fig. 1. "OD_{800}" = 0.20. 30 min experiment. 100 % initial activity = 39.4 % P_{org}.

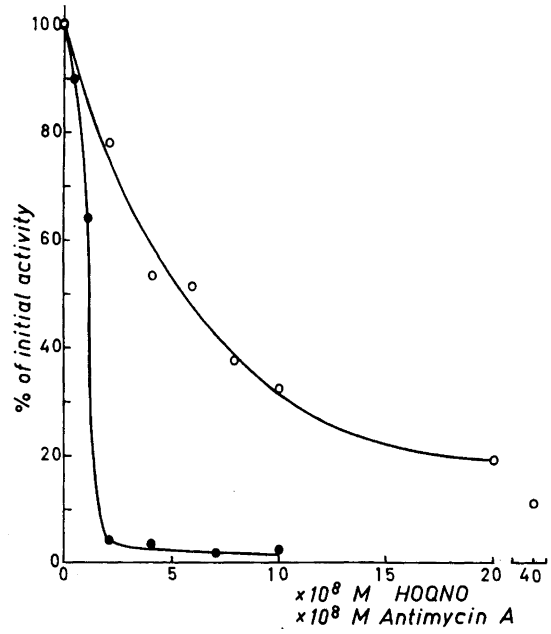

Fig. 4. Effect of antimycin A and HOQNO on LIP. Experimental details as in Fig. 1. ● = antimycin A, ○ = HOQNO. For ●, "OD_{800}" = 0.28, 100 % initial activity = 56.3 % P_{org}. For ○, "OD_{800}" = 0.32, 100 % initial activity = 62.0 % P_{org}. 30 min experiment. "Chromatophores" fraction.

The effect of two well known agents that uncouple oxidative phosphorylation in mitochondria, DNP and dicumarol, is shown in Fig. 6. Comparatively high concentrations were required to obtain an inhibitory effect on LIP. Dicumarol was much more efficient than DNP in this system.

Oligomycin A and gramicidin, which inhibit oxidative phosphorylation in animal mitochondria, also strongly inhibit LIP in extracts of *Rhodosprillium rubrum* (Figs. 7 and 8). LIP in spinach chloroplasts is inhibited by gramicidin [13,14] but not by oligomycin A in the concentrations tested [14].

DISCUSSION

A certain similarity between the mitochondrial system for oxidative phosphorylation and the system for LIP in *R. rubrum* has been indicated by the work of several investigators [1-4]. It is in the flavin and cytochrome regions that common denominators in the electron carrier systems have been suggested [3,4]. The nature of the reactions at both the reducing and the oxidizing ends of the mitochondrial electron transport chain is, however, not shared by the chromatophore system. This is shown by the fact that amytal, which inhibits electron transport in phosphorylating mitochondria between DPNH

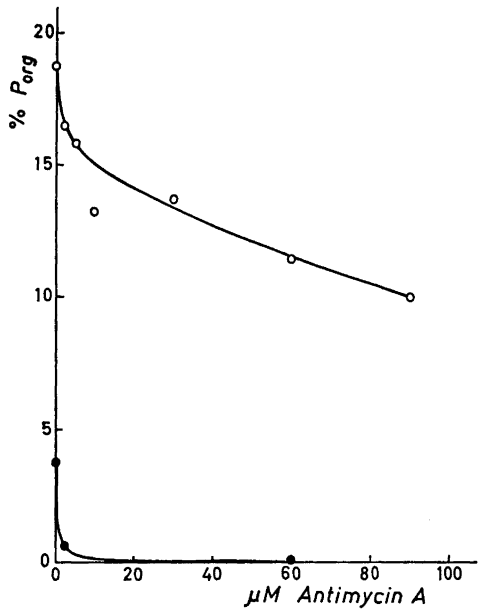

Fig. 5. Effect of antimycin A on PMS-stimulated LIP. Experimental details as in Fig. 1. O = PMS-stimulated, ● = without PMS, which curve is given as a comparison. The PMS-concentration, where added, was 3.3×10^{-4} M. "OD_{800}" = 0.50. 6 min experiment with PMS, 20 min without PMS (the values given are recalculated for 6 min assuming linearity).

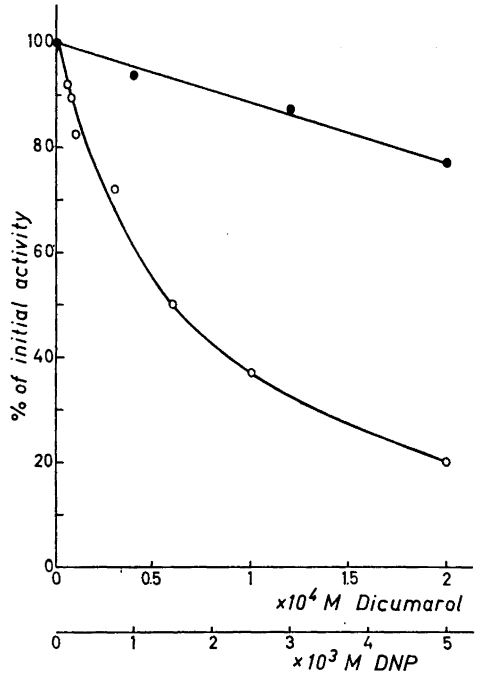

Fig. 6. Effects of dicumarol and DNP on LIP. Experimental details as in Fig. 1. O = dicumarol, ● = DNP. For O, "OD_{800}" = 0.47, 100 % initial activity = 47.5 % P_{org}, 30 min experiment. For ●, "OD_{800}" = 0.20, 100 % initial activity = 10.9 % P_{org}, 20 min experiment.

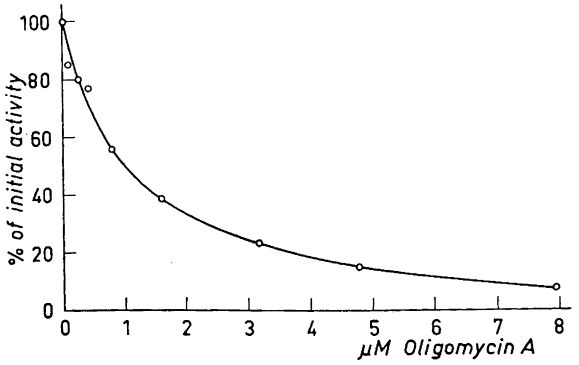

Fig. 7. Effect of oligomycin A on LIP. Experimental details as in Fig. 1. "OD_{800}" = 0.48, 100 % initial activity = 18.8 % P_{org}, 20 min experiment. "Chromatophores" fraction.

Table 1. Effects of NaN_3 and KCN on LIP. Experimental details as in Fig. 1. "OD_{800}" = 0.54, 20 min experiment.

KCN or NaN_3 concentration M	% P_{org} KCN	% P_{org} NaN_3
—	34.8	34.8
$\times 10^{-4}$	33.0	34.9
$\times 10^{-3}$	35.5	36.1
3×10^{-3}	33.1	39.9
5×10^{-3}	31.9	34.0

and flavin [15], as well as cyanide and azide, which both inhibit at the cytochrome a_3-level, have no similar effect on LIP in *R. rubrum* (Fig. 3 and Table 1).

The concentrations of antimycin A and HOQNO which were needed to inhibit LIP in *R. rubrum* (Fig. 4) were found to be almost the same as those inhibiting electron transport in mitochondrial systems [16,6]. This supports the previously formulated concept [12] that an identical or at least very similar electron carrier is acted upon in both systems.

DNP and dicumarol are "uncoupling agents" in mitochondrial oxidative phosphorylation, *i.e.* they inhibit the phosphorylation but not the respiration. In order to inhibit LIP in extracts of *R. rubrum* much higher concentrations of dicumarol and especially DNP were required than those necessary for uncoupling of the mitochondrial system [17]. It is therefore doubtful whether the inhibitory effect in the bacterial system is due to a similar mechanism as that in mitochondria. It may be appropriate to mention that at high concentration of DNP [18] and dicumarol [19] electron transport itself is inhibited in mitochondrial systems.

Also gramicidin and oligomycin A interfere with oxidative phosphorylation in animal mitochondria. Gramicidin is an "uncoupling agent" [17] and oligomycin A an inhibitor of both phosphorylation and oxidation reactions [20]. The effect of oligomycin A on respiration is especially strong with DPN-linked substrates and has been assumed to involve the inhibition of a phosphoryla-

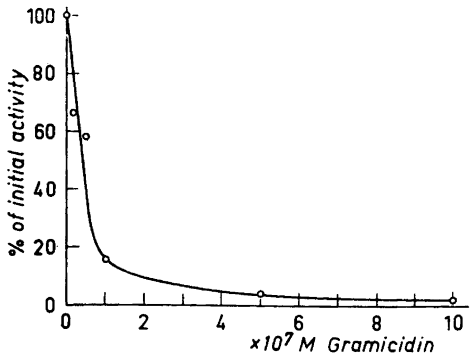

Fig. 8. Effect of gramicidin on LIP. Experimental details as in Fig. 1. "OD_{800}" = 0.20, 100 % initial activity = 13.3 % P_{org}, 20 min experiment.

tion reaction to which the respiration is obligatorily coupled [20]. The fact that both gramicidin and oligomycin A inhibited the LIP is an indication that the phosphorylation reactions proper in bacterial LIP share qualities with those of the oxidative phosphorylation reactions in animal mitochondria with respect to the mechanism of coupling between electron transport and phosphate esterification and to the nature of a reaction involved in phosphate transfer.

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