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Inhibition of the energy conservation reactions of *Rhodospirillum rubrum* by Dio-9

The antibiotic Dio-9, an inhibitor of mitochondrial oxidative phosphorylation[1] has also been shown to be a potent inhibitor of chloroplast photophosphorylation[2]. Similarly, a number of compounds have been shown to be inhibitors of both oxidative phosphorylation and bacterial photophosphorylation[3]. In view of these facts, and as a continuing effort to elucidate the mode of action of Dio-9 we have initiated a series of studies on the action of this antibiotic on the partial reactions of photophosphorylation catalyzed by chromatophores of *Rhodospirillum rubrum*. This note presents some preliminary findings in this study.

R. rubrum cells (strain S-1) were grown anaerobically in the light[4] and chromatophores were prepared from them by grinding with sand[5]. Photophosphorylation and the $^{32}P_i$–ATP exchange reaction was measured according to the procedure of HORIO *et al.*[6]. The transhydrogenase was assayed according to KEISTER AND YIKE[7]. ATPase was assayed by the procedure of PULLMAN *et al.*[8] replacing Tris acetate buffer with glycylglycine. Bacteriochlorophyll was determined by the method of CLAYTON[9].

Dio-9 is an effective inhibitor of photophosphorylation supported by succinate or phenazine methosulfate (50 % inhibition at 22 μg Dio-9 per ml). Since phenazine methosulfate by-passes one phosphorylation site[12] it would appear that Dio-9 acts at both phosphorylation sites in bacterial chromatophores. The $^{32}P_i$–ATP exchange catalyzed by this preparation is less sensitive to Dio-9. The exchange was inhibited 50 % by 36 μg Dio-9 per ml. The inhibitory effects of Dio-9 were dependent upon the concentration of antibiotic and not on the concentration of bacteriochlorophyll. By comparison, oxidative phosphorylation catalyzed by rat-liver mitochondria was inhibited 50 % by 10 μg Dio-9 per ml (ref. 1), whereas about one-tenth of this amount is required for inhibition of photophorphorylation in spinach chloroplasts[2].

The ATP and pyrophosphate (not shown) driven transhydrogenase catalyzed by *R. rubrum*[7] is inhibited by Dio-9 to the same extent as photophosphorylation. On the other hand, the light-driven transhydrogenase is less sensitive and with different

TABLE I

TITRATION OF DARK ATPASE ACTIVITY WITH CCCP

ATPase activity determined as outlined in Fig. 1.

CCCP (10^{-7} *M*)	*ATPase* (*μmoles P_i/mg bacteriochlorophyll per h*)	*ATPase* (*% of control*)
0	56	100
3	135	241
10	260	465
30	347	625
60	294	525
120	189	338
200	91	163

Abbreviation: CCCP, *m*-chlorocarbonylcyanide phenylhydrazone.

preparations the maximum degree of inhibition observed varies from 30 to 75 % at concentrations sufficient to inhibit almost completely the ATP-driven transhydrogenase or photophosphorylation. The sensitivity of the ATP driven reaction to Dio-9 is highly reproducable.

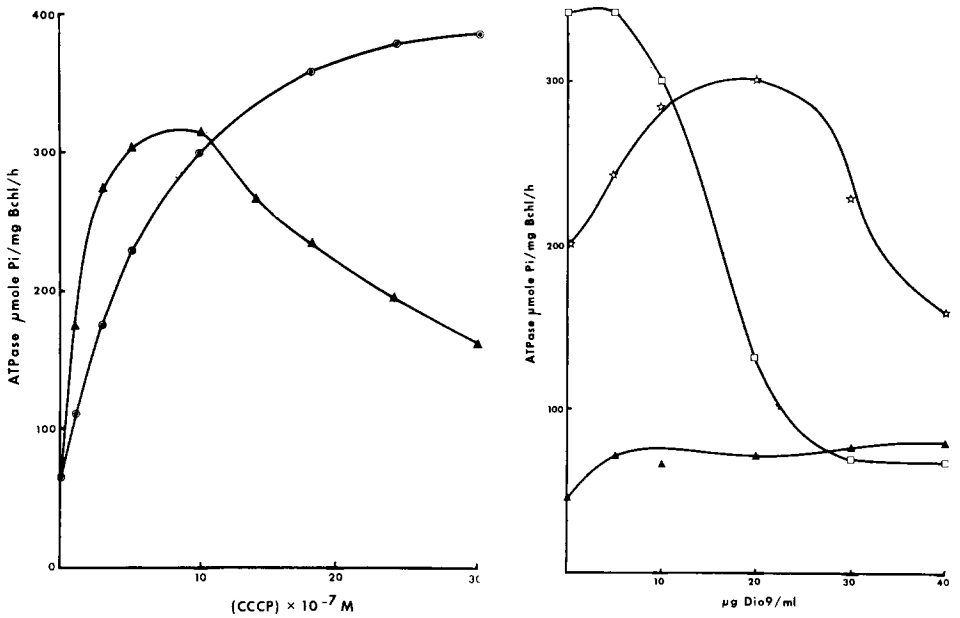

Fig. 1. Effect of Dio-9 on CCCP-stimulated dark ATPase. Reaction medium: 45 mM glycylglycine, 3.7 mM **ATP**, 3.7 mM $MgCl_2$, 18.5 mM phosphoenolpyruvate, 0.03 mg Boehringer pyruvate kinase (3.75 units), 40 μg bacteriochlorophyll (Bchl), pH 7.8, in a total volume of 1.0 ml at 30°. ◉, CCCP; ▲, CCCP with 20 μg Dio-9.

Fig. 2. The synergistic action of Dio-9 on the CCCP-stimulated chromatophore dark ATPase. ATPase activity determined as outlined in Fig. 1. ▲, CCCP concn. = 0; ✲, CCCP concn. = $6 \cdot 10^{-7}$ M; □, CCCP concn. = $3 \cdot 10^{-6}$ M.

The dark ATPase activity of isolated chromatophores is stimulated by 2,4-dinitrophenol[10] and, as shown in Table I, is stimulated as much as six-fold by low concentrations of *m*-chlorocarbonylcyanide phenylhydrazone (CCCP). Above $3 \cdot 10^{-6}$ M CCCP the stimulated ATPase activity is inhibited. In the presence of Dio-9 inhibition by CCCP is seen at a much lower concentration of the uncoupling agent (Fig. 1).

As shown in Fig. 2, ATPase is stimulated only slightly by Dio-9 in the absence of uncoupling agents. However, in the presence of $6 \cdot 10^{-7}$ M CCCP, the addition of low concentrations of Dio-9 markedly potentiated ATPase activity (Fig. 2). Similar results are observed when Dio-9 is incubated with the chromatophores together with concentrations of 2,4-dinitrophenol insufficient for maximum stimulation of ATPase. An analogous potentiation of 2,4-dinitrophenol-stimulated ATPase by laurylamine has recently been reported for rat-liver mitochondria[11].

The potentiation of the 2,4-dinitrophenol- or CCCP-stimulated ATPase by Dio-9 is a unique reaction among the known influences of inhibitors or uncouplers of energy transfer. This effect of Dio-9 may reflect a structural modification of an

enzyme system facilitating the approach of the uncoupling agents to an otherwise restricted site. This conclusion is supported by two observations. First, the total maximum ATPase (Dio-9 *plus* uncoupler) never exceeds that obtained with the uncoupler alone. Secondly, higher concentrations of Dio-9 or the uncoupling agent result in inhibition of the ATPase activity. At maximum CCCP concentration Dio-9 inhibits without prior stimulation. When the ATPase activity of rat-liver mitochondria is measured in the presence of a regenerating system for ATP, Dio-9 stimulates ATPase. Inhibition is observed at higher concentrations of the antibiotic[13]. The stimulation of ATPase activity in the intact rat-liver mitochondrial system can be correlated with a large amplitude mitochondrial swelling[14]. The possibility that Dio-9 may affect the bacterial chromatophore structure is currently under investigation. On the other hand, the relatively strong Dio-9 inhibition of the ATP- or pyrophosphate-driven transhydrogenase as compared to its partial and variable inhibition of the light driven reaction suggests a more direct effect of the inhibitor on phosphate transfer reactions. This suggestion is amplified by the magnitude of the inhibitory effect of Dio-9 on photophosphorylation in the absence of any direct stimulation of ATPase activity.

As a working hypothesis, we assume that Dio-9 acts as an inhibitor at the oligomycin-insensitive site described by BALTSCHEFFSKY AND VON STEDINGK[3] and our approach is directed towards the resolution of possible Dio-9 sensitive coupling factors in this system.

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