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PHENAZINE METHOSULFATE MEDIATED PHOTOINACTIVATION OF SOME ENERGY LINKED REACTIONS IN RHODOSPIRILLUM RUBRUM

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SUMMARY: Preillumination of Rhodospirillum rubrum chromatophores in the presence of phenazine methosulfate, under non-phosphorylating conditions results in an irreversible inhibition of the energy transduction. Protection against photoinhibition was provided during the preillumination when a continous dissipation of energy is provoked by the simultaneous photoreduction of NAD^+. The results are interpreted as indicating that the photoinactivation is produced by an accumulation of the energized form of the membrane. Different conformational forms of the ATPase complex are supposed to be responsible for the reversibility or irreversibility of the inhibited state.

INTRODUCTION:

It was earlier shown by Slooten et al. (1) that there is an irreversible inhibition of photophosphorylation and of the uncoupler-activated ATPase in Rhodospirillum rubrum thylakoids under strong light and PMS* and in the absence of substrates of photophosphorylation. The inhibition occurs after a certain degree of energization has been reached since carbonyl cyanide m-chlorophenylhydrazone prevents against inhibition. A similar situation was observed in chloroplasts inhibited by N-ethylmaleimide and in the presence of light (2,3).

Some investigators like Boyer (4) and Slater (5) believe that conformational changes needed for phosphorylation are a direct consequence of electron transport. Clear evidence for conformational changes of CF_1 attached to the membranes, during energization, was obtained by Ryrie and Jagendorf (6,7) in chloroplasts, using hydrogen exchange techniques.

The phenomenon of light dependent PMS inhibition of photophosphorylation has been tentatively described by Slooten et al. (1) as being produced by an energy dependent conformational change which is responsible for either photoinactivation or photophosphorylation when ADP and Pi are present.

We show here that the PMS photoinactivation may indeed be produced by an excessive accumulation of an energized form of the membrane since experimental conditions under which energy utilization is known to occur (other than photophosphorylation) also prevent against photoinactivation.

Abbreviations used: PMS, Phenazine methosulfate; Bchl, Bacteriochlorophyll.

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MATERIAL AND METHODS:

Rhodospirillum rubrum strain S1 was grown as previously described (8). The chromatophores were prepared in glycyl-glycine and Mg^{+2} as described (9).

Photophosphorylation

The incubation mixture used for photophosphorylation contained in 1.0 ml: 30 µmol Tricine pH 8.0; 3.7 µmol MgCl; 2.0 µmol ADP; 2.0 µmol Pi (specific activity 1.5×10^6 cpm/µmol). Other additions are described where needed. Incubation was for 2 min at 30°C under a light energy at the surface of the tubes of 2.5×10^5 erg/cm^2. sec, which is saturating for photophosphorylation. Photophosphorylation activity was measured as described before (10).

Energy linked NAD^+ reduction

ATP supported or light induced NAD^+ reduction was carried out under anaerobic conditions as described before (11) in the presence of 50 µM PMS.

Post-illumination ATP synthesis

Post-illumination ATP synthesis was carried out as described by Gromet-Elhanan et al. (12, 13) in the presence of KSCN as the permeant anion.

Other analytical procedures

Bchl was determined using a millimolar extinction coefficient of 75 as described by Clayton (14).

Radioactivity was determined in a liquid scintillation spectrometer by measuring the Cerenkov radiation of the radioactive phosphate (15).

RESULTS AND DISCUSSION:

Figure 1 shows the time course of the photoinhibition of photophosphorylation in the presence of PMS. It is interesting to notice that under the present conditions there is a lag during which no inhibition occurs. In some of the experiments an activation of the photophosphorylation is observed up to 20% over the control. Undoubtly these initial variations are due to a poising of the redox state of the electron carriers, thus facilitating electron transport. This is at variance with the results of Slooten et al. (1) in which they observe 50% inhibition occuring after few seconds of preillumination and typically 70% maximum inhibition.

We observe that the photoinactivation reaches its maximum after 2 minutes of preillumination and thereafter it comes to a plateau level which varies between experiments 40 to 50%, with PMS concentrations ranging between 50 to 200 µM.

It has been earlier described in chloroplasts that sulfate, N-ethylmaleimide and permanganate (3, 16-18) are reagents that upon energization of the membranes, inhibit energy transduction up to 50%. McCarty et al. (2) suggest that these 50% effects might correlate with the fact that EDTA easily removes only 50% of the CF_1 and subsequent addition of Dicyclohexylcarbodiimide restore photophosphorylation. No similar data are available for membranes of photosynthetic bacteria.

We have also measured post-illumination ATP synthesis in the presence of KSCN and it was similarly inhibited after 2 minutes of preillumination.

It was shown before (1) that in the photoinactivated membranes the proton uptake capacity was unimpaired, clearly revealing that the utilization of the

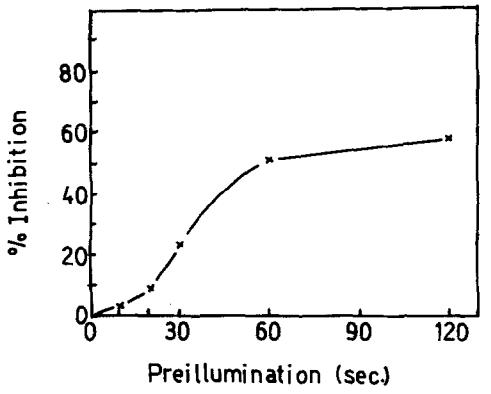

Figure 1: Kinetics of the photoinactivation of photophosphorylation in the presence of 50 µM PMS. All conditions are as described under Materials and Methods.

Table 1. Photoinactivation of energy driven NAD^+-reduction in the presence of succinate.

Pretreatment of membranes	NAD^+ reduction (umol NADH formed/h.mg Bchl)	
	ATP supported	light induced
None	8.0	25.9
50 µM PMS illuminated	0.0	11.3

energized state of the membrane was inhibited by the photoinactivation and that the electron transport was occuring at normal rates.

Table 1 shows the effect of the preillumination on another energy-driven reaction, namely the light induced or the ATP supported NAD^+ reduction in the presence of succinate. It is interesting to notice that while the light-driven reaction was inhibited only 50%, the ATP supported reaction was 100% inhibited, indicating that no energization of the thylakoid membrane is produced by using ATP. Thus the most likely candidate to account for the photoinactivations observed, is the ATPase complex. The present results do not allow us, however, to discern whether the coupling factor component or the membrane portion is inhibited (19). If the photoinhibition was prevented by the presence of Mg^{+2}, Pi and ADP by increasing the turnover of the coupling factor enzyme (1), some energy consumming reactions might provide protection against photoinactivation. Ta-

Table II. A two stage experiment on the effect of NAD^+ + Succinate on the photoinactivation of photophosphorylation.

Pretreatment of the membrane	Addition to the incubation mixture after pretreatment	LIP Percent activity
First stage		
A. None	50 µM PMS+Succ.	100
B. None	50 µM PMS+NAD^++Succ.	100
C. 3 min.Preill.+50 µM PMS+ Succ.	None	36
D. 3 min.Preill.+50 µM PMS+ NAD^++Succ.	None	27
Second stage		
Membrane A. recovered after LIP and washed.	50 µM PMS+Succ.	100
Membrane B. recovered after LIP and washed.	50 µM PMS+NAD^++Succ.	100
Membrane C. recovered after LIP and washed.	50 µM PMS+Succ.	42
Membrane D. recovered after LIP and washed.	50 µM PMS+NAD^++Succ.	84

The control membranes used in the second stage are usually 20-25% less active than the corresponding ones used in the first stage.
LIP: Light induced phosphorylation was 350 µmoles ATP/h. mg Bchl for the PMS mediated reaction. The ADP and Pi were added after each pretreatment 2 sec. before the light was turned on.
A light intensity of 2.5×10^5 erg/cm^2 . sec at the surface of the tubes containing the reaction mixture was used during preillumination and photophosphorylation.

ble II shows the effect of NAD^+ and succinate on the photoinhibition of photophosphorylation. Only 16% inhibition was detected in the presence of those substrates, when the preillumination was carried out in the presence of 50 µM PMS and NAD^+ + succinate. In the control, in which NAD^+ was omitted during the preillumination again an inhibition around 50% was observed. There is no obvious explanation to account for these results and it is particularly difficult to understand the transient inhibition of photophosphorylation in the preilluminated samples containing NAD^+ + succinate and their return to normal values after centrifugation (Table II lines 4 and 8). One might assume that there is more than one configuration involved in the energy dependent conformational change of the coupling factor. Some of these configurations would indeed have an inhibited ATPase complex. However they would still be capable of returning to the "ground" state, keeping intact their capacity to be energized again by light.

In this respect it should be pointed out that Ryrie and Jagendorf (6,7) have demonstrated that the presence of ADP + Pi inhibit the light induced tritium exchange in chloroplasts. This might indicate that either different sizes or different parts of the CF_1 molecule may be available for the hydrogen exchange in the absence or presence of the substrates of phosphorylation. This provides a clear example on the possibility of existence of different configurations of the CF_1 protein in chloroplasts.

It is evident that the photoinactivation system described here, may be used as a tool to elucidate the conformational changes that are linked to energy transduction.

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