PMS PHOTO-INHIBITION IN *RHODOSPIRILLUM RUBRUM* MEMBRANES IN THE PRESENCE OF PERMEANT ENTITIES AFFECTING EITHER THE $\Delta\psi$ OR THE ΔpH COMPONENTS OF THE PROTONMOTIVE FORCE

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1. Introduction

Preincubation of *Rhodospirillum rubrum* chromatophores in the light and in the presence of 50 μM PMS was shown [1,2] to produce an inhibition of photophosphorylation. The inhibition was prevented either in the presence of phosphate + ADP [1] or when a dissipation of the energized state is induced by another energy-utilizing reaction, namely NAD^+ reduction [2].

More than one configuration was postulated [2] to be involved in the energy-dependent conformational change which accounts for the transient photoinhibition produced by PMS in the presence of NAD^+ and succinate.

The chemiosmotic theory [3–6] postulates that the electrochemical proton gradient generated during electron transport accounts for a coupled ATP synthesis. On the other hand it was postulated [7,8] that conformational changes are necessary steps in the phosphorylation process and are directly linked to electron transport. Both in chloroplasts [9–15] and chromatophores isolated from photosynthetic bacteria [16] there have been reports that connect membrane energization to conformational changes. In the presence of different permeant ions the electric ($\Delta\psi$) and chemical (ΔpH) components of the protonmotive force ($\Delta\mu H^+$) have been shown [17] to be interconverted while the $\Delta\mu H^+$ remains rather constant.

Abbreviation: PMS, phenazine methosulfate

In order to ascertain whether the composition of the energized form of the membrane is related to its sensitivity to PMS photo-inhibition, we have tested the capacity of this substance to inhibit the light-energized membrane under different conditions favoring the production of either the $\Delta\psi$ or the ΔpH components of the protonmotive force.

2. Materials and methods

Rhodospirillum rubrum strain S1 cells were grown as in [18] and the chromatophores were prepared and washed as in [19]. Photophosphorylation was measured as in [19] in the presence of 50 μM PMS. Radioactivity was determined by measuring the Cerenkov's radiation [20] of the sample in a liquid scintillation spectrometer. $^{32}P_i$ uptake was measured according to [21].

Post-illumination ATP synthesis was measured according to [22,23], in the presence of either 1 μM valinomycin + 100 mM KCl added in the light stage or in the dark, or in the presence of 10 mM NaSCN.

Bacteriochlorophyll was measured in an acetone–methanol mixture according to [24].

3. Results and discussion

Table 1 shows the effect of PMS on post-illumina-

Table 1
Effect of preillumination on post-illumination ATP synthesis

Additions light stage	Illumination	Additions dark stage	Activity (nmol ATP/mg Bchl)
SCN^- + PMS	10 s	ADP + P_i	27.2
SCN^- + PMS	20 s	ADP + P_i	24.8
SCN^- + PMS	30 s	ADP + P_i	21.4
SCN^- + PMS	1 min	ADP + P_i	16.7
SCN^- + PMS	2 min	ADP + P_i	13.3
PMS	3 min		
then SCN^-	2 min	ADP + P_i	12.6
Val + KCl + PMS	10 s	ADP + P_i	35.3
Val + KCl + PMS	20 s	ADP + P_i	49.0
Val + KCl + PMS	30 s	ADP + P_i	63.6
Val + KCl + PMS	1 min	ADP + P_i	61.3
Val + KCl + PMS	2 min	ADP + P_i	64.0
PMS	3 min		
then + Val + KCl	2 min	ADP + P_i	5.0
PMS at pH 6.0	2 min	ADP + P_i + Val + KCl	22.8
None at pH 6.0	2 min	ADP + P_i + Val + KCl	35.0

Unless otherwise indicated the pH during the light stage was 7.7 for SCN^- and 7.3 for valinomycin + KCl. In all cases 20 μg Bchl were used. The light intensity was 2×10^6 erg cm^{-1} s^{-1}. The final PMS concentration was 50 μM. All other conditions were as in section 2

tion ATP synthesis. It shows that when SCN^- is the permeant anion, pre-illumination of the membrane with PMS produces a strong inhibition. When valinomycin + K^+ were used in these experiments, pre-illumination of the membrane in the presence of PMS produces a similar inhibition. However, a difference is readily apparent; thus, when PMS + valinomycin + K^+ were present during pre-illumination, no inhibition was detected, whereas for SCN^- under the same conditions, the dark ATP synthesis activity was strongly dependent on the pre-illumination time.

The reason for such a difference is not obvious considering that under both conditions a stimulation of the H^+ uptake [22,23] is produced as a consequence of the ability of both substances to decrease the membrane potential. Moreover, both of them stimulate post-illumination ATP synthesis [22,23].

When valinomycin + K^+ were added in the dark stage and thus inducing a potassium diffusion potential [23], an inhibition of dark ATP synthesis was again observed by pre-illuminating the membrane, at pH 6.0, in the presence of PMS. This result seems to be at variance to that in [1] on the pH dependency of PMS photo-inhibition.

In order to clarify this point, we have further measured the photo-inhibition produced by PMS at different pH values by measuring the phosphorylating capacity, at pH 8.0. It is observed that pre-incubation of the membrane, at pH 6.0, in the absence of phosphate and in the presence of PMS, produces an inhibition of the phosphorylating capacity similar to that observed at higher pH values, whereas addition of 2 mM phosphate protects against the inhibition by light + PMS during the low pH treatment which agrees with [1]. The presence of phosphate at pH > 6.8 does not protect against photo-inhibition.

These experiments and those in [1,2] show that pre-illumination with PMS probably inhibits the energy transfer process, and the marked difference existing between SCN^- + PMS and valinomycin + K^+ + PMS may be interpreted assuming that although energization under either condition produces a similar p.m.f. value it results from a different combination of the $\Delta\psi$ and the ΔpH components. Valinomycin + K^+ had

Table 2
Effect of pre-illumination with PMS in the presence of salts on the phosphorylating capacity of membranes

Additions during pre-illumination			% inhibition
None		+ PMS	60
NH_4Cl	10 mM	+ PMS	10
NaSCN	10 mM	+ PMS	25
NaCl	10 mM	+ PMS	63

Pre-illumination time was 1 min. PMS in all cases was 50 μM. The PMS-supported phosphorylation activity of the control was 600 μmol ATP formed/h × mg Bchl

a double additive effect [22,23] creating a light-induced ΔpH and a superimposed potassium diffusion potential in the dark, whereas in the presence of SCN^- the $\Delta\mu H^+$ was almost totally the result of a light-induced ΔpH. It must also be remembered that the $\Delta\mu H^+$ produced in the presence of valinomycin + K^+ is lower than that measured in the presence of other lipophilic anions [25,26]. Thus the $-Z\Delta pH$ term does not compensate the p.m.f. due to a lower electron transport produced by a low pH. Therefore, if the extent of PMS inhibition is directly proportional to the energization extent, a lower inhibition in the presence of valinomycin + K^+ is to be expected.

Table 2 shows the effect of pre-illumination with PMS in the light and in the presence of salts known to induce either $\Delta\psi$ or ΔpH [17]. Light energization in the presence of NH_4Cl which is known to induce a $\Delta\mu H^+$ formed almost totally by $\Delta\psi$ and SCN^- that acts by shifting the $\Delta\mu H^+$ to 100% ΔpH, do not allow photo-inhibition. On the other hand the addition of NaCl does not modify the amount of photo-inhibition produced in the absence of any added ions. Similarly 10 mM NaCl does not induce any change in the light-induced $\Delta\mu H^+$ [17], whereas addition of either 10 mM NH_4Cl or SCN^- will decrease the p.m.f. (15–20%) and allowing therefore a smaller energization and consequently, a smaller PMS photo-inhibition.

Thus, it is clear that light in the presence of PMS is the only condition inducing a sufficiently large membrane energization and perhaps a configurational change of the CF facilitating PMS inhibition. These results do not prove that a structural change of the CF-protein is the only cause for the PMS inhibition.

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