PHOTOREACTIVATION OF MANGANESE CATALYST IN PHOTOSYNTHETIC OXYGEN EVOLUTION

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Manganese functions in photosystem II, the O_2-evolving process of photosynthesis (Cheniae, et al, 1966; Eyster, et al, 1958; Gerhardt, 1966; Kessler, 1955; Kessler, et al, 1957; Pirson, 1937; Richter, 1961; Spencer, et al, 1960; Spencer, et al, 1961) and addition of manganese to the growth medium of deficient cells results in the restoration of the process (Cheniae, et al, 1966; Eyster, et al, 1958; Gerhardt, 1966). This restoration in Scenedesmus cells was shown to be independent of de novo protein or chlorophyll synthesis but dependent upon light (Cheniae, et al, 1966). With this alga the rate of restoration proved relatively slow, and not conducive to kinetic analysis. Experiments are reported on the restoration of photosynthesis and the Hill-reaction in Mn-deficient cells of the blue-green alga Anacystis.

Experimental.

Anacystis nidulans was cultured at 35° in Roux bottles on medium C of (Kratz, et al, 1955) gassed with 3% CO_2 in air. Light intensity was 1200 foot candles. Manganese deficiency was obtained by strict exclusion of the element from the medium, using glass-distilled, de-ionized water, Spec-Pure chemicals for trace elements and glassware cleansed with 4N HCl. Cells were harvested by centrifugation after 48 hours of growth starting with 20 µl packed cell volume per 600 ml of medium, ending at 225 and 1600 µl for deficient and normal cells, respectively. Packed cell volume was determined by centrifugation of a suitable aliquot in a hematocrit tube for 30 min. at 1100 xg. Chlorophyll determinations were made following 80% acetone extraction (Arnon, 1949).

Polarographic rate measurements were made at 23° with a teflon covered Pt electrode, which sampled a 1 ml lucite reaction vessel (1 cm light path) provided with a rotating magnetic stirrer. Response of the O_2 measurement was 2-5 seconds. Light from a 750 watt lamp at 100 volts filtered through 12 inches of water and an OG-3 (Schott) filter was focussed onto the vessel. Light saturation was just obtained at full intensity. Intensity was varied using neutral density filters. Photosynthesis was measured using cells equivalent to 10 μg Chl. a suspended in 0.08M $NaHCO_3$ saturated with 5% CO_2 in air. Hill reaction activity was measured as outlined in Table I.

Results.

In Fig. 1 the rates of respiration and photosynthesis are compared for normal and Mn-deficient Anacystis. Expressed per unit chlorophyll, respiration of the deficient alga (Curve 1) is several fold greater than that of normal

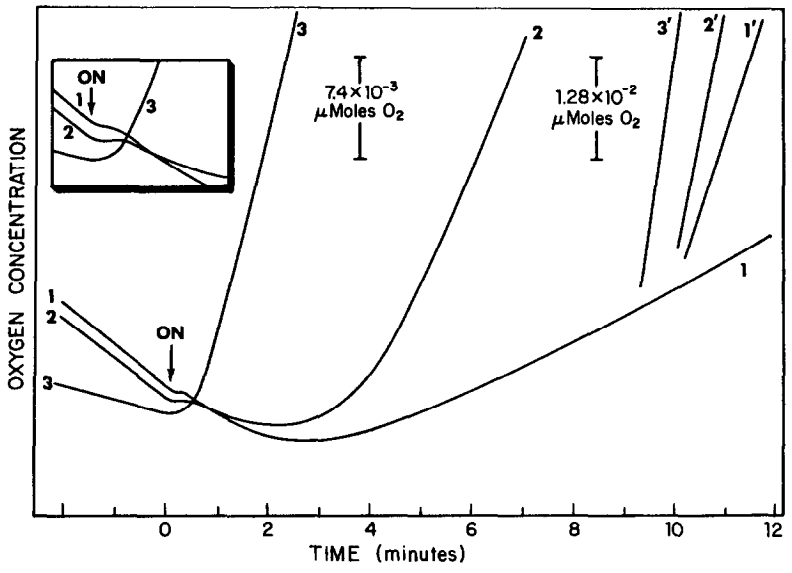

Figure 1 - Assay mixture contained Anacystis cells equivalent to 10 μg Chl_A suspended in 0.08M $NaHCO_3$ saturated with 5% CO_2 in air containing 0.005M EGTA except where $MnCl_2$ (0.02 μmoles) was added. This concentration of EGTA had no inhibitory effect on normal cells nor initial rates of deficient cells and thus was used to complex manganese ions in the assay mixture. Curves 1, 2 and 3 represent rates of deficient cells, deficient cells plus Mn^{+2}, and normal cells, respectively all at low intensity. The respective rates at saturating intensity are designated as 1', 2' and 3'.

Anacystis cells (Curve 3). The chlorophyll content of the deficient cells was 2-3 times lower, thus there is no significant difference between the respiration rates of the two types of cells if expressed per unit dry weight. At low intensities a marked (4.6 fold) difference in the rate of photosynthesis (uncorrected for respiration) was observed between the normal and deficient cells. At saturating intensities (Curves 1' and 3'), this difference was less pronounced, but clearly present. Curves 2, 2' illustrate the effect of addition of Mn^{++} (0.02 μmoles) to the deficient cells in dark 5 minutes prior to illumination and the subsequent change in rate of photosynthesis upon illumination. After ~ 1 minute of illumination a change in rate was observed which attained a steady rate after 5 minutes. The rate of respiration did not change during or after reactivation. Under the conditions of this experiment complete return to a normal cell activity was not attained; however, as will be reported elsewhere complete return on a chlorophyll a basis, to normal saturation rate and quantum

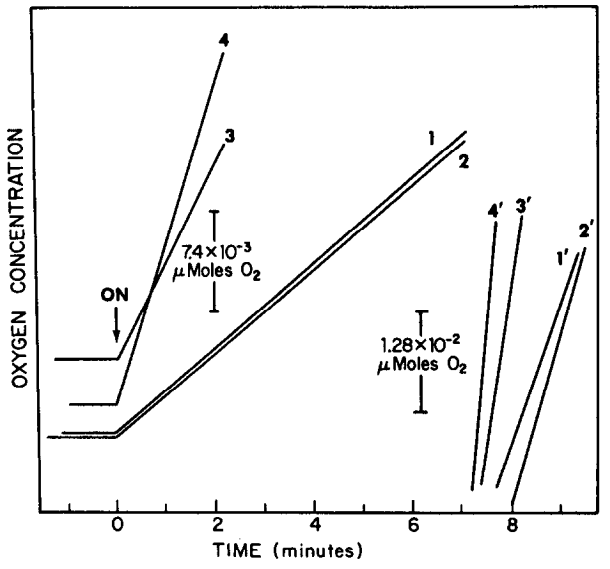

Figure 2 - Assay mixture contained in micromoles: Phosphate buffer, pH 6.7, 126; FeCn, 4.0, benzoquinone, 0.1; KCN, 1.0 and Anacystis cells equivalent to 10 μg Chl_a in total volume of 1.0 ml. The final pH of mixture was 7.0. Curves 1 and 1' are rates obtained with Mn deficient cells at 5.5% I and 100% I, respectively. Curves 2 and 2' with Mn deficient cells in reaction mixture plus 0.02 μmoles $MnCl_2$; curves 3 and 3' with Mn deficient cells preilluminated 5 minutes at 5.5% I in the presence of phosphate buffer and 0.02 μmoles $MnCl_2$ only; curves 4 and 4' with normal cells.

yield can be attained with different conditions in comparable time-periods.

Figure 2 shows that Mn-deficiency affects the Hill-reaction in much the same way as it does photosynthesis. The deficient cells (Segments 1 and 1') showed considerably lower rates than the normal cells (Segments 4 and 4'). If quinone was added prior to illumination of deficient cells preincubated in dark with Mn^{+2}, no increase of the rate occurred at low intensity (Segment 2) and only a slight increase in saturating light (Segment 2'). If, however, deficient cells were preilluminated in the presence of Mn^{+2} but in the absence of quinone, KCN and FeCN and subsequently assayed for Hill-activity, a significantly improved activity was observed at both intensities (Segments 3 and 3').

TABLE I

EFFECT OF PREINCUBATION MIXTURE ON RESTORATION OF HILL ACTIVITY

Type of Cells (Anacystis)	Omissions from Light Preincubation	Hill Activity (μMoles O_2/mg Chl_A/hour)	
		5.5% I	100% I
Normal	None	57.0	359
Mn Deficient	None	10.5	78.9
Mn Deficient	- Mn^{+2}	9.7	81.0
Mn Deficient	-Benzoquinone	47.3	294
Mn Deficient	-FeCN, Benzoquinone	45.1	280
Mn Deficient	-FeCN, Benzoquinone, KCN	47.1	283

The reaction mixture for Hill assays contained the following in micromoles: K Phosphate buffer, pH 6.75, 120; FeCN, 4; Benzoquinone, 1.0; KCN, 1.0; $MnCl_2$, 0.02 and 10μg Chl_a in a total volume of 1.0 ml. For light preincubations (10 min. at 5.5%I) the reaction mixture was the same except for omissions noted. Cells incubated with Mn^{+2} were preincubated 20 minutes in darkness before additions for preincubation in the light. Duration of assay of Hill activity at the low and high intensity was 5 and 2 minutes, respectively.

As shown in Table I reactivation was inhibited by benzoquinone. For complete inhibition one-tenth the quinone concentration used in experiments (Fig. 2 and Table I) sufficed. Ferricyanide (FeCN) by itself had no effect. This agent, however, supported rates of only 5 percent of quinone - Hill

reaction rate and might not penetrate the cells. KCN, at a concentration which abolished photosynthesis did not affect reactivation, which indicates that CO_2-fixation or products thereof are not involved in the reactivation process.

The reactivation effect was specific for Mn^{+2} salts ($MnCl_2$, $MnSO_4$). Preincubation of deficient cells in the light with Fe^{+2}, Fe^{+3}, Mg^{+2}, Zn^{+2} or Co^{++} at equal or five fold higher concentration than the optimal concentration of Mn^{+2} had no effect.

DCMU, an inhibitor of O_2 evolution (Bishop, 1958), in concentrations which completely inhibited Hill activity fully inhibited the photoreactivation (Table II). Also, 50% inhibition of the two processes was obtained at equal concentrations of DCMU. Removal of the DCMU by repeated washings of the cells restored photoreactivation as it did the Hill reaction, showing that the inhibitory effect

TABLE II

EFFECT OF DCMU ON PHOTO-REACTIVATION OF

MANGANESE DEFICIENT ANACYSTIS

DCMU Concentration (Molar x 10^{-6})	Inhibition (percent)
0	0
0.01	19.0
0.004	32.3
0.40	55.3
1.0	77.4
10	92.1

Three milliliter cell suspensions (5 μg Chl_a/ml) in 25 ml erlenmeyer flasks were preincubated in darkness for 30 minutes in growth medium containing 1.8 x 10^{-5}M $MnSO_4$. DCMU was added where noted, and the suspensions then were illuminated for 2 minutes at an intensity of 260 foot-candles. Following repeated washings with 0.02M phosphate, pH 6.75 containing 0.001M EDTA, an aliquot (4 μg Chl_a) was assayed for Hill activity (see Fig. 2) at saturating intensities. Normal cell controls with DCMU, after the repeated washings, showed 80% of activity of cells without DCMU. Before and after reactivation the deficient cell rates were 29 and 73% of the normal cell rates, respectively.

of DCMU is reversible in the two equally sensitive processes. Thus, it appears that the photoreaction of system II is an "autocatalytic" process as is also suggested by the shape of the restoration curves in Fig. 1. Preliminary action spectra appear to be consistent with the conclusion that the photoprocess is driven by system II.

The effect of Mn deficiency on the induction phase of photosynthesis (Fig. 1, insert) mimics the effect of long periods of anaerobiosis reported for many photosynthetic tissues (Rabinowitch, 1956). Very similar recordings were observed for Chlorella under anaerobic conditions by (Spruit & Kok, 1956). In aerobic Mn-deficient cells this induction is not observed after only slight (~ 20%) photo-restoration of deficient cells by Mn^{+2}. Similarly, the induction phase of anaerobic cells (Rabinowitch, 1956; Spruit, et al, 1956) is markedly decreased following an illumination period. It is suggested that the long term anaerobiosis effect is the result of inactivation of the manganese catalyst in photosynthetic O_2-evolution.

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Summary and Conclusions.

Restoration of photosynthesis in Mn deficient Anacystis cells after addition of Mn^{+2} ion shows an absolute requirement for light. Photoreactivation does not require operation of the CO_2 reduction cycle, but does depend upon activity of photosystem II, and is inhibited by benzoquinone.

We conclude that Mn^{+2} is bound to its apoenzyme in a dark thermal reaction but is as such inactive as a catalyst in O_2-evolution; activation of the complex occurs in a photochemical reaction, sensitized by photosystem II. Possibly bound-Mn^{+2} is oxidized to a higher valency state in the photoreaction.

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