ON THE LIGHT-DEPENDENT REACTIVATION OF PHOTOSYNTHETIC ACTIVITY BY MANGANESE

Bernt Gerhardt* and Wolfgang Wiessner

Pflanzenphysiologisches Institut, Abt. Biochemie der Pflanzen, Universität Göttingen, Germany

Received August 10, 1967

The cultivation of the blue-green alga Anacystis nidulans in a manganese deficient medium leads to a decreased photosynthetic activity (Richter, 1961). Photosynthesis and quinone Hill reaction are restored immediately in intact cells without any lag phase by adding catalytic amounts of manganese (Gerhardt, 1967). In this case the process of reactivation of photosynthetic activity by manganese takes place at the same time photosynthesis is measured. However, it has been shown recently that the process of reactivation and measurement of photosynthetic activity can be separated (Gerhardt, 1967). The cells must be freezedried after incubation with manganese and the photosynthetic activity has to be determined in lyophilized cells (Gerhardt and Trebst, 1965; Gerhardt and Santo 1966). Application of this method has it made possible to examine various conditions for the restoration of photosynthetic activity by manganese independent from a concomitant determination of photosynthetic oxygen evolution. Thus it could be demonstrated that the reactivation of photosynthetic

*Present address, Purdue University, Department of Biological Sciences, Lafayette, Indiana 47907.

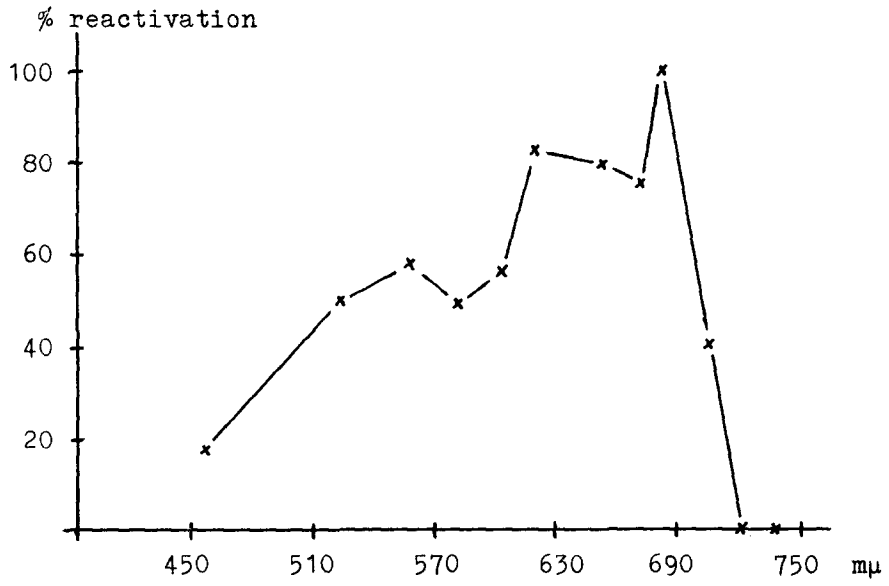

Figure 1. Relative action spectrum of the reactivation of Hill reaction by Mn-deficient lyophilized Anacystis.

1 μmol $MnCl_2$/0.1 mg chlorophyll/ml was added to Mn-deficient algae. Then the algae were irradiated for 5 min with monochromatic light obtained from a Sun-Gun lamp (Sylvania) with interference filters (Schott and Gen., Mainz, Germany). White light = 100% reactivation; dark control = 0%. The algae were lyophilized in 5% sucrose after irradiation. The Hill reaction of the lyophilized algae was determined manometricaly. Reaction mixture (μmoles): Tris buffer pH 7.6, 80; $MgCl_2$, 40; p-benzoquinone, 0.5; ferricyanide, 20; and 0.2 mg chlorophyll. Total volume, 3 ml. 35000 lux; 20° C; N_2; reaction time 8 min.

---

activity by manganese requires light (Gerhardt, 1967; similar results were obtained by Cheniae and Martin, 1967). Oxygen or CO_2 are not needed, showing that complete photosynthesis or respiration are not necessary for the process of reactivation by manganese.

Table 1. Activity of the Hill reaction in Mn-deficient lyophilized Anacystis after irradiation of intact cells with nearly equal intensities of light at λ 680 mμ or 719 mμ in the presence of manganese.

Wavelength [mμ]	Absorbed energy during incubation with Mn^{2+} before freezedrying	μatome O evolved after 8 min light in freezedried cells
Dark	---	5.6
719	$1.56 \cdot 10^3$ erg/sec	5.5
680	$1.27 \cdot 10^3$ erg/sec	8.6

Light absorption by the algae was 60% measured in an integrating sphere. The light source was calibrated against the radiant energy of a standard lamp (Eppley Lab., Inc., Newport, Rhode Island) measured with a large surface bolometer (H. Röhrig, Berlin, Germany). For experimental details see Figure 1.

This communication shall present data on the dependency of the reactivation of the quinone Hill reaction in manganese deficient Anacystis nidulans on wavelength and light intensity.

The action spectrum of the reactivation of the quinone Hill reaction is given in Figure 1. It resembles essentialy the action spectrum of photosynthesis of blue-green algae (Emerson and Lewis, 1942; Haxo and Blinks, 1950). It shows a maximum at λ 680 mμ which is the main peak of absorption of chlorophyll attributed to light reaction II of photosynthesis (for references see Witt, 1965). A second maximum at λ 617 mμ corresponds to the main peak of absorption of phycocyanin. At wavelengths longer than λ 680 mμ the reactivation decreases significantly. No reactivation can be observed beyond λ 719 mμ.

Table 2. Dependency of the reactivation of the Hill reaction by manganese in Mn-deficient Anacystis on light intensities and time of exposure at λ 680 mμ.

Exposure time	Absorbed energy during incubation with Mn^{2+} before freezedrying	% reactivation of the Hill reaction in freezedried cells
5 min	$0.80 \cdot 10^2$ erg/sec	11
	$1.20 \cdot 10^2$	23
	$1.57 \cdot 10^2$	50
	$2.96 \cdot 10^2$	70
	$4.25 \cdot 10^2$	85
25 min	$1.26 \cdot 10^2$ erg/sec	96

Reactivation by white light = 100%. Light absorption by the algae was 60%. For experimental details see Figure 1.

Table 3. Activity of the Hill reaction in Mn-deficient Anacystis after incubation of intact cells with manganese in the dark or light.

Incubation with Mn^{2+}	μatome O evolved a) intact cells	b) lyophilized cells
none	3.8	4.6
5 min in the dark	7.4	5.1
5 min in white light	7.1	8.5

Incubation with manganese: 0.15 μmoles $MnCl_2$/0.2 mg chlorophyll. Algae lyophilized in 5% sucrose. Reaction mixtures (μmoles): a) Phosphate buffer pH 7.0, 100; p-benzoquinone, 0.5; ferricyanide, 20; and 0.1 mg chlorophyll. b) Tris buffer pH 8.0, 80; $MgCl_2$, 40; p-benzoquinone, 0.5; ferricyanide, 20; and 0.2 mg chlorophyll. Total volume, 3 ml. 35000 lux; 20° C; N_2; reaction time 8 min.

Table 1 contains data on the reactivation of the quinone Hill reaction at two wavelengths, λ 680 mμ and 719 mμ, characteristic for the absorption of photosystem II (and I) and I alone. Even though the intensities of the absorbed light energy are nearly equal the exclusive excitation of the photosystem I (λ 719 mμ) does not lead to a reactivation. Excitation of photosystem II (λ 680 mμ) is necessary.

Table 2 presents data on the dependency of the reactivation on the intensity of absorbed light energy at λ 680 mμ. A good agreement between the degree of reactivation and the intensity of absorbed light energy can be observed. At low light intensities which do not reactivate the Hill reaction at short time of exposure the reactivation can be completed extending the time of irradiation.

The necessity of light for the reactivation of photosynthetic activity is not due to a light dependent manganese uptake (Table 3). If Anacystis cells are preincubated with manganese in the light or in the dark and washes twice, subsequent illumination still leads in both cases to reactivated cells. This indicates that manganese had been taken up also in the dark and reactivation had occured simultaneously with photosynthesis. If, however, the cells are freezedried after preincubation with manganese the reactivation of photosynthetic activity takes place only when the algae have been preincubated with manganese in the light. So far reactivation of manganese deficient Anacystis by manganese in a freezedried preparation was not possible.

Photophosphorylation is not necessary for the reactivation of the quinone Hill reaction in manganese deficient Anacystis by manganese (Table 4). The uncoupler of photophosphorylation

Table 4. Photosynthetic O_2-evolution by Mn-deficient Anacystis in presence of the uncoupler undecyl-benzimidazole (UDB).

	µatome O evolved	
	without $MnCl_2$	+ 0.15 µmoles $MnCl_2$
Photosynthesis:		
uninhibited	3.1	5.0
inhibited by 40γ UDB/3 ml	0.7	1.0
Hill reaction:		
uninhibited	3.4	5.2
inhibited by 40γ UDB/3 ml	3.3	5.9

Photosynthesis: Reaction mixture: 100 µmoles phosphate buffer pH 7.0 and 0.1 mg chlorophyll. Total volume, 3 ml. Air-5%CO_2; 20° C; 35000 lux; reaction time 15 min.
Hill reaction: Reaction mixture (µmoles): Phosphate buffer pH 7.0, 100; p-benzoquinone, 0.5; ferricyanide, 20; and 0.1 mg chlorophyll. Total volume, 3 ml. 35000 lux; 20° C; N_2; reaction time 8 min.

---

undecyl-benzimidazole (Büchel, Röchling, Baedelt, Gerhardt, and Trebst, 1967) does not inhibit the reactivation of the quinone Hill reaction at concentrations sufficient to stop photosynthesis.

In summary the experiments presented in this communication indicate the necessity of the photosystem II in order to restore photosynthetic activity in manganese deficient Anacystis by manganese. Probably Mn^{2+} has to be oxidized in a light dependent process. Experiments to determine the nature of the oxidized manganese are in progress.

Acknowledgements. This research was supported by the Deutsche Forschungsgemeinschaft. The excellent technical assistance of Miss R. Santo is gratefully acknowledged.

References

Büchel, K.H., Röchling, H., Baedelt, H., Gerhardt, B., and Trebst, A., Z. f. Naturforschg. 22b, 535 (1967).

Cheniae, C.M., and Martin, I.F., In Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symposia in Biology, No. 19, p. 406 (1967).

Emerson, R., and Lewis, C.M., J. gen. Physiol. 25, 579 (1942).

Gerhardt, B., Ber. Dtsch. Bot. Ges. 79, (63) (1967).

Gerhardt, B., and Santo, R., Z. f. Naturforschg. 21b, 673 (1966).

Gerhardt, B., and Trebst, A., Z. f. Naturforschg. 20b, 879 (1965).

Haxo, F.T., and Blinks, L.R., J. gen. Physiol. 33, 389 (1950).

Richter, G., Planta 57, 202 (1961).

Witt, H.T., Angew. Chem. 77, 821 (1965).