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THE METHYL VIOLOGEN-CATALYZED MEHLER REACTION AND CATALASE ACTIVITY IN BLUE-GREEN ALGAE AND *CHLAMYDOMONAS REINHARDI*

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SUMMARY

1. The methyl viologen-catalyzed Mehler reaction was investigated in intact cells of five species of blue-green algae and *Chlamydomonas reinhardi*.

2. In the presence of methyl viologen, all the blue-green algae except *Anabaena flos-aquae* show a light-dependent O_2 consumption as well as a post-illumination O_2 evolution. The rate of O_2 consumption is stimulated by 1 mM KCN, an inhibitor of catalase, but the dark O_2 evolution becomes suppressed.

3. *A. flos-aquae* shows a light-dependent methyl viologen-catalyzed O_2 uptake which is not affected by 1 mM KCN. Furthermore, there is no release of O_2 in the dark following illumination.

4. With *C. reinhardi*, the cells do not show any net O_2 exchange during or after illumination. Addition of 1 mM KCN, however, results in an immediate O_2 uptake in the light.

5. Based on the mechanism postulated for the Mehler reaction in isolated chloroplasts, it was deduced that the differences in the kinetics of the O_2 exchange catalyzed by methyl viologen reflect differences in the endogenous catalase activity in these algae. Cells of *A. flos-aquae* are deficient in catalase activity whereas those of the other blue-green algae possess catalase, although at low activity. *C. reinhardi*, on the other hand, has high catalase activity *in vivo*.

6. These findings are corroborated by results obtained from O_2 electrode measurements of catalase activity in cell-free extracts of these algae.

7. The possible roles of catalase in algae and the implications of these results are also discussed.

INTRODUCTION

The interaction between molecular O_2 and the photosynthetic electron transport pathway was first investigated by MEHLER[1] and MEHLER AND BROWN[2]. Using broken chloroplasts they demonstrated that O_2 was reduced to H_2O_2 upon illumina-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PBQ, *p*-benzoquinone.

tion. Subsequent investigations confirmed[3] and extended this observation and it is now known that the rate of O_2 uptake by illuminated chloroplasts could be stimulated by the addition of auto-oxidizable compounds[4–7].

So far, the Mehler reaction has been studied mainly in isolated chloroplast fragments. Little is known about the existence and the magnitude of this reaction in intact cells[8]. Whether this light-dependent O_2 uptake occurs *in vivo* in the presence of an exogenous auto-oxidizable compound has still not been investigated. If it occurs, then the rate of the net O_2 uptake should depend partly upon the endogenous catalase activity of the cells.

Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) has always been assumed to be present in all plants although its distribution in the plant kingdom has never been properly studied[9]. PATTERSON AND MEYERS[10] reported, in an abstract, that among a few species of blue-green algae examined catalase is present only in *Anacystis nidulans*. HONEYCUTT AND KROGMANN[11] failed to detect any catalase activity in a photosynthetic lamellae preparation of *Anabaena variabilis* although the same preparation could photoreduce O_2 to H_2O_2. In view of the finding that H_2O_2 is a product of the interaction between molecular O_2 and the reduced form of certain components of the electron transport pathway[11,12], it becomes important to establish whether catalase is indeed absent from blue-green algae.

In this paper, we present results of investigations on the kinetics of O_2 exchange catalyzed by methyl viologen in intact cells of blue-green algae and *Chlamydomonas reinhardi*. The results will be discussed in relation to the catalsae activity in these organisms.

MATERIALS AND METHODS

Organisms and conditions of culture

Five species of blue-green algae and one species of green algae were used in these experiments.

Anabaena flos-aquae (1444), *Anabaena spiroides* (1552), *Phormidium luridum* var. *olivacea* (426), *Plectonema boryanum* (594) and *Plectonema calothricoides* (598) were obtained as bacteria-free cultures from the culture collection of algae at Indiana University. Cells of these algae were grown at 27–29° in 5-l flasks each of which contained 3 l of the growth medium. The latter was prepared according to ALLEN AND STANIER[13] and was supplemented with 0.15 % $NaNO_3$ (w/v). The medium was stirred vigorously by means of a magnetic stirrer. Cultures were illuminated continuously with two banks of daylight fluorescent lamps placed parallel to each other on opposite sides of the flasks. The light intensity on each side of the flask was about 1000 ft candles as measured with a Weston Illumination Meter (Model 756).

Chlamydomonas reinhardi (137c) was a gift from Prof. R. P. Levine (The Biological Laboratories, Harvard University). Cells of this alga were cultured at 27–29° in 300-ml portions of liquid growth medium contained in 500-ml erlenmeyer flasks. The growth medium was either a minimal one containing inorganic salts or the same medium fortified with 0.2 % (w/v) sodium acetate. The composition of the minimal inorganic medium has been described[14]. Cells grown in the minimal medium are said to be phototrophic and those grown in the fortified medium mixotrophic. Continuous illumination of the cultures was provided by two banks of daylight fluorescent lamps

at the same intensity as described above. The culture flasks were constantly agitated on a reciprocal shaker operated at a speed of 100 strokes/min with a linear displacement of 1 inch.

The methyl viologen–Mehler reaction and the p-benzoquinone–Hill reaction in intact algae

The Mehler reaction catalyzed by methyl viologen was carried out with intact cells suspended in 10 mM potassium phosphate (pH 7.0) containing 2 mM methyl viologen. The cell suspension was contained in a lucite cell (1.50 ml) and was continuously mixed with a magnetic stirrer.

The change of O_2 concentration in the lucite cell was measured with a conventional Clark polarographic electrode (Yellow Springs Instrument Co., Model 5331) which was connected to an electron unit (Yellow Springs Instrument Co., Model 53). The rates of O_2 exchange were displayed on a Servogor potentiometric recorder (Type 9031) operated at a sensitivity of 100 mV for full scale deflection of the recorder chart. The chart speed of the recorder was set at 1 cm/min.

The algal cells were irradiated with white light from a Leitz slide projector equipped with a tungsten filament lamp (500 W, 240 V). The light intensity at the position of the lucite cell was about 11000 ft candles as measured by a Weston Illumination Meter (Model 756). The temperature during the experiments was about 28°.

The Hill reaction with *p*-benzoquinone (PBQ) as the Hill oxidant was also carried out with intact cells suspended in 10 mM potassium phosphate (pH 7.0) The final concentration of PBQ in the reaction mixture was 2 mM. The rate of O_2 evolution was measured with the oxygen electrode and under the same experimental conditions described above. A Beckman scale expander (73490) was connected to the potentiometric recorder to provide for off-set voltage. By adjusting the zero suppression control knob on the scale expander panel, the recorder pen can be brought to any desired position without affecting the calibration of the recorder.

Chlorophyll concentration in blue-green algae was determined in 90 % methanol extract according to the method of OGAWA AND SHIBATA[15]. Chlorophyll concentration in *C. reinhardi* was measured by the procedure of ARNON[16].

Methyl viologen and PBQ (analytical reagent grade) were purchased from Sigma and Koch-Light, respectively. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) was a gift from E.I. du Pont de Nemours and Co., Inc.

Preparation of cell-free extracts

Liquid cultures of algae grown under conditions described above were used to prepare cell-free extracts. Each culture was checked for possible bacterial contamination by inoculating 1-ml aliquots onto two or three nutrient agar plates. The plates were then incubated in the dark at 37° and the culture was considered to be free of contamination if no bacterial growth was detected on the plates after 48 h.

Cells were harvested toward the end of the exponential phase of growth by filtration. They were washed twice with 1 mM potassium phosphate (pH 7.0) and resuspended in 50 mM potassium phosphate (pH 7.0) to a concentration of approx. 20–30 % (v/v).

The cell suspension was sonicated for 4 min in a Raytheon 10 kcycles/sec sonic oscillator (Model DF-101) maintained at 4°. The sonicate was centrifuged at 500 × *g* for 5 min. The pellet was discarded, and the supernatant was centrifuged at 27000 × *g*

for 30 min. All centrifugations were performed at about 4°. The 27000 × *g* pellet was resuspended immediately in 50 mM potassium phosphate (pH 7.0). This fraction as well as the supernatant were assayed for their catalase activities.

Protein concentration was determined by the procedure of LOWRY *et al.*[17].

Assay of catalase activity

The catalase activity was assayed by a slight modification of the procedure described by GREGORY[18]. Aliquots of the supernatant or the pellet fraction were diluted with suitable amounts of 50 mM potassium phosphate (pH 7.0). This diluted crude enzyme preparation was contained in a lucite cell (1.50 ml) and was constantly stirred by a magnetic stirrer. The reaction was started by the addition of 20 μl of 0.4 M H_2O_2 with a microsyringe (Hamilton, U.S.A.). The H_2O_2 (0.4 M) solution was prepared by diluting 30 vol. H_2O_2 (analytical reagent grade) with 50 mM potassium phosphate (pH 7.0). The solution was standardized by titration against standard $KMnO_4$ in the presence of H_2SO_4.

The amount of O_2 released in the lucite cell was monitored at 28° by a conventional Clark polarographic electrode as described earlier for the measurement of the PBQ–Hill reaction. The chart speed of the recorder was set at 4 cm/min.

Usually the rate of the O_2 evolution was measured for about 1 min before the addition of H_2O_2 and 2 min after the reaction was started. The rate of O_2 evolution due to the catalase activity was linear for at least 1 min. There was generally no change in O_2 concentration without the addition of the substrate. Addition of H_2O_2 to the 50 mM potassium phosphate (pH 7.0) alone resulted in a slow rate of O_2 evolution, which was always subtracted from that obtained in the presence of the crude enzyme preparation.

In each experiment, the catalase activity was assayed with at least five different protein concentrations. A straight line was always obtained when the rates of O_2 evolution were plotted against protein concentrations in the reaction mixture. The specific activity of catalase in the sample was calculated from the gradient of this straight line.

RESULTS

The methyl viologen–Mehler reaction in blue-green algae

Fig. 1a shows the time course of the O_2 exchange catalyzed by 2 mM methyl viologen, in whole cells of *P. calothricoides*. Upon illumination of the cells, there is a rapid O_2 uptake and this is followed by a slower rate of O_2 evolution in the dark after illumination. The light-dependent O_2 uptake is completely eliminated by the addition of 5 μM DCMU, indicating the requirement of Photosystem II in this process (Fig. 1b).

The above results are interpreted according to the mechanism postulated for the Mehler reaction in isolated chloroplasts[1]. Similar to its mode of action in isolated chloroplasts[6,7], methyl viologen functions as a Hill oxidant *in vivo* and is reduced by Photosystem I in the light. Since Photosystem I is dependent upon Photosystem II as the ultimate source of electrons, the photoreduction of methyl viologen is inhibited by DCMU which is a Photosystem II inhibitor. Subsequent reoxidation of the reduced methyl viologen results in the formation of H_2O_2. With chloroplast preparations deficient in catalase activity, H_2O_2 is not dismutated, and this leads to a net uptake

of O_2. In intact cells, on the other hand, the H_2O_2 produced is liable to attack by the endogenous catalase. Under conditions in which the catalase activity is high, the H_2O_2 is completely dismutated giving no net O_2 exchange either during or after illumination. However, when the catalase activity is low, as in the case of *P. calothricoides*, a light-dependent O_2 consumption is observed. The O_2 released by the cells in the dark is due to the action of catalase on the H_2O_2 accumulated during the preceding light period.

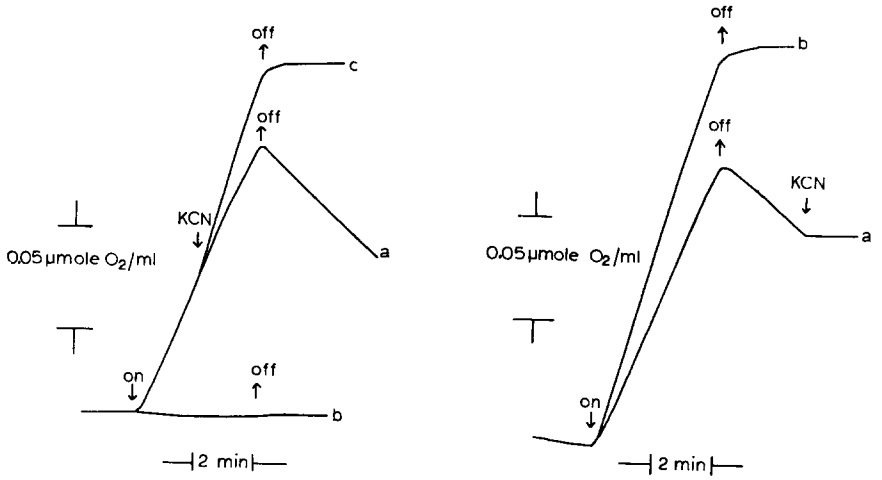

Fig. 1. Kinetics of the O_2 exchange catalyzed by methyl viologen in *P. calothricoides*. Chlorophyll concentration was 5 µg/ml. Cells were incubated with methyl viologen in the dark for 15–20 min before the experiment was started. Other experimental conditions have been described under MATERIALS AND METHODS. The rate of O_2 exchange was measured for approx. 2 min before the light was turned on. The cells were kept in the light for 4 min. An upward deflection of the recorder tracing indicates O_2 consumption whereas a downward deflection indicates O_2 evolution. a, no addition; b, *plus* 5 µM DCMU added before the experiment was started; c, *plus* 1 mM KCN added in the light as indicated.

Fig. 2. Kinetics of the O_2 exchange catalyzed by methyl viologen in *P. calothricoides*. Experimental conditions are the same as those described in Fig. 1. a, *plus* 1 mM KCN added in the dark following illumination as indicated; b, *plus* 1 mM KCN added before the experiment was started.

If these interpretations are correct, then the slow rate of O_2 evolution in the dark should be abolished by inhibitors of catalase. Furthermore, the rate of the light-dependent O_2 consumption catalyzed by methyl viologen should be enhanced by the addition of a catalase inhibitor provided the latter does not interfere with the photosynthetic electron transfer reactions.

As illustrated in Fig. 2a, the dark O_2 evolution following illumination is completely inhibited by 1 mM KCN. The same inhibitory effect was obtained with 1 mM NH_2OH, 1 mM NaN_3 and 1 mM semicarbazide, all known inhibitors of catalase[19]. Fig. 2b shows that the rate of the light-dependent O_2 uptake is increased by the addition of 1 mM KCN in the dark preceding illumination. This stimulatory effect of KCN is also obtained if it is added during illumination (Fig. 1c). Under these conditions the post-illumination O_2 evolution is suppressed (Figs. 1c and 2b).

The methyl viologen–Mehler reaction was also investigated in four other blue-green algae: *P. boryanum*, *P. luridum*, *A. spiroides*, and *A. flos-aquae*. All these algae,

with the exception of *A. flos-aquae*, produced results similar to those obtained with *P. calothricoides* indicating that the endogenous catalase activity is low in these organisms.

A typical time course of the methyl viologen-catalyzed O_2 exchange in intact cells of *A. flos-aquae* is similar to that of *P. calothricoides* treated with KCN. There is no release of O_2 in the dark following illumination. In addition, the rate of O_2 uptake in the light is not affected by 1 mM KCN added either before or during the light period. These findings strongly suggest that catalase activity is deficient in this particular alga.

The rates of the light-dependent O_2 uptake catalyzed by methyl viologen in the blue-green algae are presented in Table I. Fig. 3 shows that, in *P. luridum*,

TABLE I

RATES OF THE LIGHT-DEPENDENT O_2 CONSUMPTION CATALYZED BY METHYL VIOLOGEN IN BLUE-GREEN ALGAE AND *C. reinhardi*

The procedure for the measurement of the light-dependent O_2 consumption has been described under MATERIALS AND METHODS. Chlorophyll concentration was 5 μg/ml for the blue-green algae and 15 μg/ml for *C. reinhardi*. Cells were incubated in the dark with 2 mM methyl viologen for 15–20 min before the experiment was started.

Organism	*Rate* (*μmoles O_2 consumed/mg chlorophyll per h*)	
	No additions	*+ 1 mM KCN*
P. calothricoides	425	553
P. boryanum	304	537
P. luridum	366	608
A. spiroides	182	491
A. flos-aquae	510	510
C. reinhardi (mixotrophic)	0	105
C. reinhardi (phototrophic)	0	98

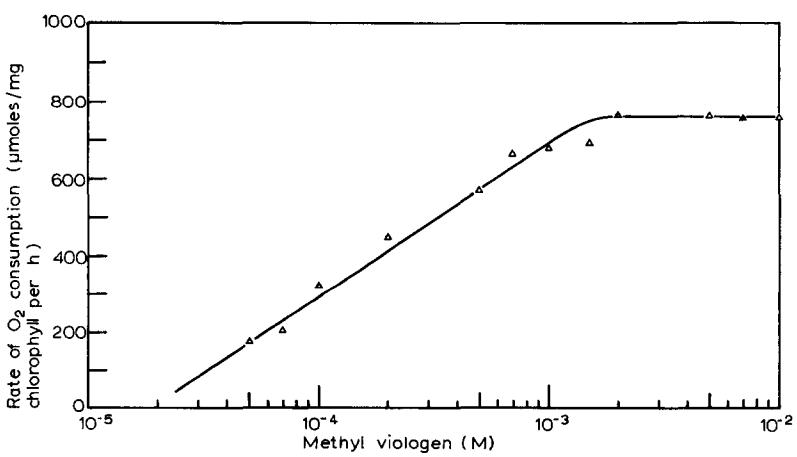

Fig. 3. The effect of methyl viologen concentration on the rate of the light-dependent O_2 consumption in *P. luridum*. Chlorophyll, 5 μg/ml; KCN, 1 mM. Each sample was incubated with the appropriate concentration of methyl viologen in the dark for about 15–20 min before illumination. Other experimental conditions can be found under MATERIALS AND METHODS.

the rate of the light-induced O_2 uptake by cells treated with KCN saturates at a methyl viologen concentration of 2 mM.

Fig. 4 compares the effects of varying light intensities on the rates of the PBQ–Hill reaction and the methyl viologen–Mehler reaction. In *A. variabilis*, SUSOR AND KROGMANN[20] found that the ferricyanide–Hill reaction saturated at about 15000 ft candles. This value is higher than that for the PBQ–Hill reaction reported here. It should be noted, however, that their experiments were performed with cell-free preparations whereas in our system intact cells were used.

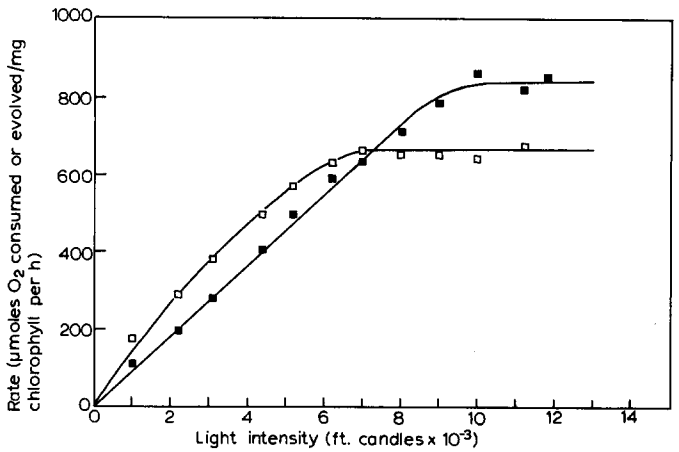

Fig. 4. Effects of varying light intensities on the rates of the methyl viologen–Mehler reaction (□—□) and the PBQ–Hill reaction (■—■) in *P. luridum*. The methyl viologen–Mehler reaction was measured as the rate of the light-dependent O_2 uptake. Chlorophyll, 5 μg/ml; KCN, 1 mM; methyl viologen, 2 mM; dark incubation period, 15–20 min. The PBQ–Hill reaction was measured as the rate of O_2 evolution. Chlorophyll, 5 μg/ml; PBQ, 2 mM. The light intensity was varied by means of a set of Wratten neutral density filters (No. 96) and metal screens. The light intensity was measured at each point with a Weston Illumination Meter (Model 756). Other experimental conditions have been described under MATERIALS AND METHODS.

The methyl viologen–Mehler reaction in C. reinhardi

In contrast to the blue-green algae described above, intact cells of *C. reinhardi* do not consume or release O_2 when they are illuminated in the presence of methyl viologen (Fig. 5a). Addition of 1 mM KCN to the illuminated cells, however, results in an immediate O_2 consumption (Fig. 5b). Light-dependent O_2 uptake is also observed when the cells are pretreated with KCN (Fig. 5c). Here again, DCMU abolishes this light-dependent O_2 uptake in KCN-treated cells (Fig. 5d). These observations suggest that the endogenous catalase activity in whole cells of *C. reinhardi* is high and is capable of dismutating all the H_2O_2 as fast as it is formed by the reoxidation of the reduced methyl viologen. Hence, no net O_2 exchange is obtained under normal conditions. However, a net O_2 consumption by the cells is observed when the endogenous catalase activity is suppressed by KCN.

Results described above were obtained with cells of *C. reinhardi* grown under mixotrophic conditions. Phototrophic cells gave similar results.

The rate of the methyl viologen–Mehler reaction is given in Table I. There is no significant difference between cells that are grown phototrophically and those grown mixotrophically.

The response of the methyl viologen–Mehler reaction toward varying light intensities is depicted in Fig. 6. The light saturation curve for the PBQ–Hill reaction is also shown for comparison.

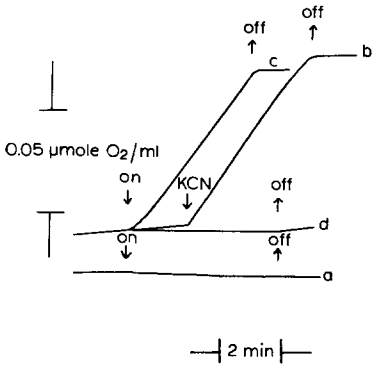

Fig. 5. Kinetics of the light-dependent O_2 uptake in *C. reinhardi*. Cells were grown under mixotrophic conditions. Chlorophyll, 15 μg/ml; methyl viologen, 2 mM. Cells were incubated with methyl viologen in the dark for 15–20 min before the experiment was started. The rate of O_2 exchange was followed for 2 min before illumination. For other experimental conditions, see MATERIALS AND METHODS and Fig. 1. a, no addition; b, *plus* 1 mM KCN added 2 min after the light was turned on; c, *plus* 1 mM KCN added just before the experiment was started; d, *plus* 1 mM KCN and 5 μM DCMU both added just before the experiment was started.

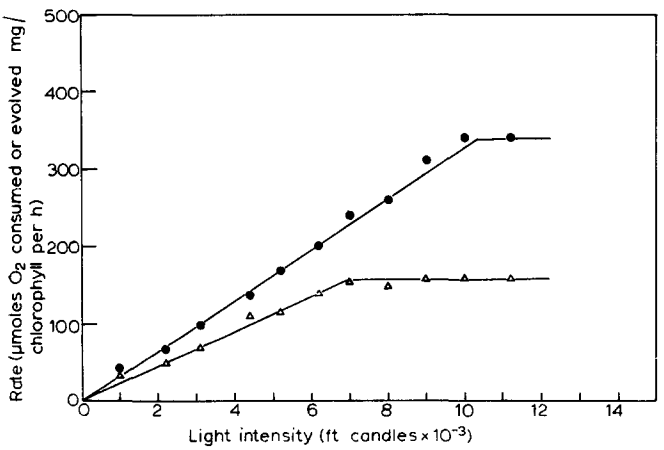

Fig. 6. Effects of varying light intensities on the rate of the methyl viologen–Mehler reaction (△—△) and the PBQ–Hill reaction (●—●) in *C. reinhardi*. Cells were cultured under mixotrophic conditions as described under MATERIALS AND METHODS. Chlorophyll concentration was 15 μg/ml. Other experimental conditions are the same as those given in Fig. 4.

Catalase activities in cell-free extracts of blue-green algae and C. reinhardi

Table II gives specific activities of catalase in cell-free extracts of five species of blue-green algae and one species of green algae. In each experiment, the catalase activity of the cell-free extract is completely abolished by the addition of the following catalase inhibitors: KCN (50 μM), NH_2OH (50 μM) and NaN_3 (50 μM). The enzyme activity also disappears after incubating the extract at 65° for about 5 min.

In the blue-green algae, a low specific activity of catalase was detected in the

TABLE II

CATALASE ACTIVITIES IN CELL-FREE EXTRACTS OF BLUE-GREEN ALGAE AND *C. reinhardi*

Procedures for the preparation of cell-free extracts and the assay of catalase activity have been described in detail under MATERIALS AND METHODS. The pellet fraction (27000 × *g* for 30 min) contains about 5–10% of the total catalase activity.

Organism	*Specific activity* (*nmoles H_2O_2 decomposed/mg protein per min*)	
	Supernatant	*Pellet*
P. calothricoides	$3.6 \cdot 10^3$	$8.0 \cdot 10^2$
P. boryanum	$9.8 \cdot 10^2$	$1.9 \cdot 10^2$
P. luridum	$4.9 \cdot 10^2$	$1.3 \cdot 10^2$
A. spiroides	$6.9 \cdot 10^3$	$6.8 \cdot 10^2$
A. flos-aquae	$0.2 \cdot 10^2$	$0.5 \cdot 10^2$
C. reinhardi (mixotrophic)	$5.6 \cdot 10^4$	$7.3 \cdot 10^3$
C. reinhardi (phototrophic)	$1.1 \cdot 10^4$	$2.0 \cdot 10^3$

pellet as well as in the supernatant after centrifugation at 27000 × *g* for 30 min. As shown in Table II, the specific activity of catalase of the former is about 4–10 times less than that of the latter. Furthermore, the pellet fraction contains only about 5–10 % of the total catalase activity found in the supernatant after the first centrifugation (500 × *g* for 5 min) which removed cell debris and unbroken cells. The remainder of the catalase activity is found in the 27000 × *g* supernatant. Therefore, the low specific activity of catalase in blue-green algae cannot be attributed to a low efficiency of enzyme extraction since if this were the case the pellet fraction would be expected to have a greater amount of catalase activity as well as a higher specific activity compared to the 27000 × *g* supernatant.

Since *A. flos-aquae* shows almost no catalase activity either in the supernatant or the pellet fraction, the possibility of an endogenous catalase inhibitor being present in this alga was tested. This was done by mixing varying amounts of the *A. flos-aquae* supernatant with a fixed amount of the supernatant from *P. luridum*. It was found that the catalase activity in the latter was not affected under these conditions. Thus the lack of catalase activity in *A. flos-aquae* is not due to the presence of a soluble endogenous inhibitor. However, the possibility of an insoluble inhibitor being bound to the photosynthetic membranes cannot be ruled out.

DISCUSSION

In this paper, we show that under appropriate conditions it is possible to demonstrate a light-dependent O_2 uptake attributable to the methyl viologen–Mehler reaction in whole cells of blue-green algae and *C. reinhardi*. The magnitude of this O_2 consumption reflects the level of endogenous catalase activity in these organisms.

The saturating methyl viologen concentration for the methyl viologen–Mehler reaction in *P. luridum* is about 2 mM. This concentration is 200 times higher than that of the same reaction in spinach chloroplasts[7]. The discrepancy can be attributed to at least two factors. First, in our system, there is a permeability barrier imposed by the living cells. Second, the methyl viologen has to be present in high enough concentration so that it can compete with the endogenous ferredoxin for electrons from Photosystem I.

Comparisons of the light intensity-response curves reveal that the methyl viologen–Mehler reaction is saturated at a lower light intensity than the PBQ–Hill reaction. This may reflect differences in the participation of the two photosystems in these partial photosynthetic reactions. The methyl viologen–Mehler reaction requires the cooperation of both photosystems whereas the PBQ–Hill reaction depends only on Photosystem II. SUSOR AND KROGMANN[20,21] noted that, in *A. variabilis*, the Hill reaction with either ferricyanide or $NADP^+$ as the oxidant saturated at a higher light intensity than the photoreduction of $NADP^+$ with reduced indophenol as the electron donor. Our results are consistent with their findings if the Photosystem I activity is rate-limiting in the methyl viologen–Mehler reaction.

Contrary to earlier reports by other workers[10,11], we found that four out of five blue-green algae examined possess catalase although at low specific activity. However, a more extensive survey is necessary before any generalizations can be made with regards to the distribution of this enzyme in blue-green algae. It must be emphasized that the O_2 electrode measurements of catalase activity reported here is much more sensitive than the conventional spectrophotometric method of catalase assay. The latter method may not permit the detection of a low specific activity of catalase in cell-free extracts of blue-green algae. PATTERSON AND MEYER[10] used both the scopoletin-peroxidase assay method and the O_2 electrode measurements to investigate catalase activity. However, it is difficult to compare their results with those reported here since details of the results and assay methods are available only in an abstract.

It is of interest to note that, although both *A. spiroides* and *A. flos-aquae* belong to the same genus, catalase activity is detected in the former but not the latter species. *P. calothricoides* and *P. boryanum*, on the other hand, both possess catalase. HONEYCUTT AND KROGMANN[11] have reported the absence of catalase activity in photosynthetic lamellae of *A. variabilis*. In our experiments, the specific activity of catalase in the pellet fraction, which contained mainly photosynthetic lamellae, is about 4–10 times less than that of the supernatant. This level of specific activity of catalase may not be detected by the spectrophotometric method of catalase assay. Therefore, whether or not catalase is lacking in *A. variabilis* is still an open question.

The specific activity of catalase in cell-free extracts of *C. reinhardi* agrees well with that reported recently by BRUIN *et al.*[22]. In their experiments the cells were cultured under phototrophic conditions.

The presence of catalase in blue-green algae and *C. reinhardi* invites speculation as to the physiological role of this enzyme. There is strong evidence that catalase in higher plants is localized in a special organelle called peroxisome[23]. During the metabolism of glycollate, it is oxidized by glycollate oxidase into glyoxylate and H_2O_2. The H_2O_2 formed is then decomposed by catalase into H_2O and O_2 (ref. 23). However, experiments with green algae[24] and *A. flos-aquae*[25] reveal that in these organisms glycollate oxidase is replaced by glycollate dehydrogenase. This seems to preclude the function of catalase in the glycollate metabolism of these organisms.

Recently, MATHIEU *et al.*[26] have reported that in spinach chloroplasts the light-induced O_2 uptake due to the Mehler reaction is stimulated by ferredoxin. Further experiments along this line demonstrate that H_2O_2 is formed as a result of interaction between molecular O_2 and reduced ferredoxin[12]. There is also evidence that the photosynthetic lamellae of *A. variabilis* are able to photoreduce O_2 to H_2O_2 (ref. 11). This photoreduction appears to be a manifestation of the ferredoxin-reducing factor

discovered earlier by YOCUM AND SAN PIETRO[27]. If this is the case, then reoxidation of the reduced form of ferredoxin-reducing factor or ferredoxin itself will lead to the formation of H_2O_2. The magnitude of these reactions could be significant at high light intensity and low CO_2 concentration. Catalase, although present at low specific activity in the blue-green algae, could still play an important role in the dimutation of H_2O_2 formed *via* these side reactions of the photosynthetic electron transport pathway.

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