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# THE DIFFERENTIAL ACTION OF METRONIDAZOLE ON NITROGEN FIXATION, HYDROGEN METABOLISM, PHOTOSYNTHESIS AND RESPIRATION IN *ANABAENA* AND *SCENEDESMUS*

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## Summary

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) at 1—2 mM levels has been shown to be a selective inhibitor of nitrogenase activity in *Anabaena*. Two constitutive hydrogenases and photosynthesis are insensitive to metronidazole at these same concentrations. At higher concentrations metronidazole inhibits photosynthesis in *Anabaena* while photoreduction and to a lesser extent photohydrogen production are retarded in *Scenedesmus*. Respiration is slightly stimulated at high metronidazole levels in both algae. The reductant source for nitrogenase in *Anabaena* and photohydrogen production and photoreduction electron transport in *Scenedesmus* are discussed. Due to the activity of metronidazole as a selective inhibitor of ferredoxin-associated processes, it should prove to be useful in $N_2$ fixation studies and in distinguishing between ferredoxin-linked reactions of different sensitivities and other activities not associated with low reduction potential components.

## Introduction

Cetain blue-green and green algae produce and consume molecular hydrogen. The $H_2$ production in blue-green algae is catalyzed by a nitrogenase which is dependent on oxidative or photophosphorylation for ATP and a light-indepen-

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DSPD, disalicylidenepropanediame; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), sodium salt; Ps-I, Photosystem I; PS-II, Photosystem II.

dent reaction for reductant [1,2]. However, rates of photohydrogen production or light-dependent $C_2H_2$ reduction are higher than the respective maximum aerobic, dark values [3—5]. The inability to obtain greater nitrogenase rates in the dark under aerobic conditions is apparently due to (1) limitations in the dark ATP production system, (2) reversible (or irreversible) inhibition of nitrogenase with $O_2$, (3) limitation of the production of reductant in the dark, or (4) reverse uptake reactions in the case of $H_2$. $H_2$ uptake in *Anabaena* has been attributed to the presence of an oxy-hydrogen type reaction [6,7] and photohydrogen uptake can be deduced from reports of $H_2$ stimulation of $C_2H_2$ reduction in the light [8—10]. $H_2$ uptake and production activities have been difficult to separate experimentally.

In the green algae $H_2$ production requires Photosystem I (PS-I) and is mediated through a hydrogenase and unaffected by uncouplers [11,12]. The source of reductant appears to be water but $H_2$ evolution may be observed in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) suggesting an additional electron donor site [13]. Light-stimulated $H_2$ uptake under anaerobic conditions results in the DCMU-insensitive photoreduction of $CO_2$ [14,15], whereas the terminal acceptor for $H_2$ in the presence of $O_2$ in the light or dark may be $O_2$ [16,17].

Metronidazole has been recognized as having antimicrobial properties since the 1950's but it was much later that its mode of action began to be elucidated [18—20]. Metronidazole is now known to interfere with ferredoxin-linked reactions and to inhibit hydrogenase activity associated with phosphoroclastic reactions and $CO_2$ reduction in photosynthesis [21—23]. The mechanism of inhibition appears to be due to alteration of normal electron transport with metronidazole as an electron acceptor. Toxic products are produced through reduction of metronidazole or as a result of the formation of superoxide and peroxide [24—27].

This paper reports the differential effects of metronidazole on the endogenous nitrogenase, hydrogenase and photosynthetic activities of two strains of *Anabaena* and compares these results to the effects on $H_2$ metabolism and photosynthesis in *Scenedesmus*.

## Materials and Methods

*Culturing conditions*

Cultures of *Anabaena cylindrica* Lemm (629) and *Anabaena* 7120 were grown on mineral medium [28] with NaCl substituted equimolarly for $NaNO_3$. Cultures were illuminated at a light intensity of approx. 8 mW/cm$^2$ from mixed fluorescent tubes and tungsten bulbs, maintained at 24—27°C, and aerated with 2% $CO_2$ in air. Cultures were maintained at a density of approx. 3 µl/ml of culture medium in a modified continuous growth apparatus [29]. Batch cultures were subcultured daily. Cell densities varied between 1.5 and 5.0 µl/ml and cultures were sampled for experimental use at approx. 4.5 µl/ml. *Scenedesmus obliquus*, strain $D_3$ was cultured heterotrophically as described previously [30].

*Amperometric measurement of $H_2$ and $O_2$*

$O_2$ and $H_2$ concentrations were followed in a 1.3 ml dual electrode cuvette

that was regulated at 27°C and similar to that described earlier [6]. $O_2$ and $H_2$ responses were calibrated with culture media or buffer in equilibrium with air and 2% $H_2$ using published solubility values [31,32]. Rates were calculated as pmol or nmol/μl per min since these units were those used in the experiments. 1 μl of *A. cylindrica* cells represents 1 μl packed cell volume and 0.5 nmol or μg of chlorophyll *a*. Illumination was provided by a tungsten filament microscope lamp or quartz-iodide source with respective incident intensities of 7.5 and 15 mW/cm$^2$ after passing through 2.5 cm of 2% (w/v) $CuSO_4$.

*Determination of $H_2$ production, photosynthesis, and respiration*

*Anabaena.* Aliquots of up to 12 ml were taken from growth tubes, pelleted, resuspended in buffer (see figure legends), transferred to 30-ml flasks and capped with serum stoppers. The flasks were evacuated three times substituting argon after each evacuation. Gas additions and cell removals were made through the serum stoppers using hypodermic needles.

In some experiments metronidazole was added as concentrated aqueous or dimethyl sulfoxide solutions. Maximum concentrations of dimethyl sulfoxide were 350 mM and it was included in all controls. Otherwise dilute aqueous solutions of metronidazole were used to inhibit cells in timed pretreatments before analysis. $O_2$-stimulated $H_2$ production was initiated in the dark by adding 0.13 ml buffer saturated with 100% $O_2$. After rates were established, light-stimulated $H_2$ production and photosynthetic $O_2$ production were recorded followed by a second dark period. Gross photosynthesis was calculated as the sum of $O_2$ production in the light and $O_2$ uptake in the dark at the same $O_2$ concentration. Respiration rates were established in the dark at specified $O_2$ concentrations.

$H_2$ uptake determinations were similar to $H_2$ production analyses. Cells were removed from batch cultures and stored in dim light on a shaker. Aliquots were concentrated and resuspended in buffer containing DCMU. Nitrogenase was inactivated by incubating cells for 10 min in 100% $O_2$ at 27°C and metronidazole was added to the cells which were then pretreated for a specified time under argon. The flasks were evacuated a final time and 3% $H_2$ and 20% $O_2$ were added with a balance of argon. The flasks were agitated to obtain equilibrium in the liquid phase and transferred to the reaction cuvette within 30 s. $H_2$ uptake rates in the presence of $O_2$ were established and haulted by the addition of KCN to 300 μM. After 1 min photohydrogen uptake was initiated by illumination from a quartz-iodide lamp.

*Scenedesmus.* Photosynthesis of *S. obliquus* was measured amperometrically using a cell concentration of 10 μl/ml in buffer. Cells treated with metronidazole were incubated with inhibitor for 1 h prior to the determination of activity. Photoreduction was measured manometrically according to the method previously described [30] and 5 μM DCMU was included to prevent deadaptation during the measurements. After a 4 h anaerobic adaptation period the cells were incubated 2 h in metronidazole prior to initiating the measurements. Photohydrogen production was determined as previously described [6,13] with cells prepared as for photoreduction. The Hill reaction was measured amperometrically with *p*-benzoquinone as the oxidant [30].

*Acetylene reduction assays*

Samples were assayed simultaneously in $C_2H_2$ reduction experiments. Cells were taken to a density of 15 $\mu$l/ml in culture media containing 20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 6.50. For light-stimulated $C_2H_2$ reduction assays 2.5 ml each of cells and aqueous metronidazole solutions were added to 30-ml flasks. The cells were rendered anaerobic as in the polarographic assays but 8% $C_2H_2$ and a balance of argon were added prior to assay. Reaction mixtures were incubated for 30 min on a shaking bath held at 27°C and supplied with tungsten light providing an intensity of 7.5 mW/cm$^2$. After the reaction period 0.5 ml samples of the gas mixtures over the cells were taken and $C_2H_4$ analysis was performed by gas chromatography (Hewlett Packard Mo. 5830 A with 18850 A terminal) with flame ionization detection. An Alltech aluminum column (6 ft X 0.125 in, Porapac R, 80—100 mesh) was used at 45°C and flow rate of approx. 30 ml/min. Conditions for $O_2$-stimulated $C_2H_2$ reduction in the dark were the same with the exception that light was excluded and 7.5% $O_2$ was included in the reaction flasks.

*Carbon fixation assays*

Reaction conditions were similar to the $C_2H_2$ reduction assays except the initial gas phase was pure argon. Immediately before the assays 0.57 ml of 53.6 mM $NaH^{14}CO_3$ with a specific activity of 9.46 $\mu$Ci/mmol was added, giving a final concentration of 5.49 mM. Reactions were stopped with 0.5 ml glacial acetic acid. The unfixed $HCO_3^-$ was dissipated and aliquots were pipetted to 2.3-cm circles of Whatmann 3 MM paper, dried, placed in scintillation vials with 5 ml of Biofluor (New England Nuclear) and counted in a Packard 2425 counter. A maximum of 1.5% of the label was incorporated into the acid-stable fraction. $Ba^{14}CO_3$ was purchased from New England Nuclear and converted to $H^{14}CO_3^-$.

## Results and Discussion

The effect of metronidazole on $H_2$ production, photosynthesis, and respiration in *A. cylindrica* is illustrated in Fig. 1. It is clear that both $O_2$-stimulated and light-stimulated $H_2$ production are severely inhibited by 2.0 mM metronidazole, whereas photosynthesis and respiration are not. However, 20 mM inhibitor treatments result in a slight stimulation of respiration and a strong inhibition of photosynthesis. It is evident that inhibition of $H_2$ production is well separated from other effects. The metroidazole concentrations used to inhibit nitrogenase selectively (Figs. 1 and 3) are similar to the levels that were effective on hydrogenases that were associated with ferredoxin-mediated electron tranasport [19]. Higher concentrations have been used to inhibit cellular photosynthetic systems [20,27].

During these analyses it was observed that the effects of metronidazole inhibition were not immediate but were progressive. Therefore, the time course inhibition of 1.0 mM metronidazole was determined (Fig. 2). In the previous experiment a 5 min pretreatment with metronidazole inhibited nitrogenase the same as that seen in Fig. 2. However, nearly complete inhibition of photohydrogen production was observed after 11 min of pretreatment whereas no

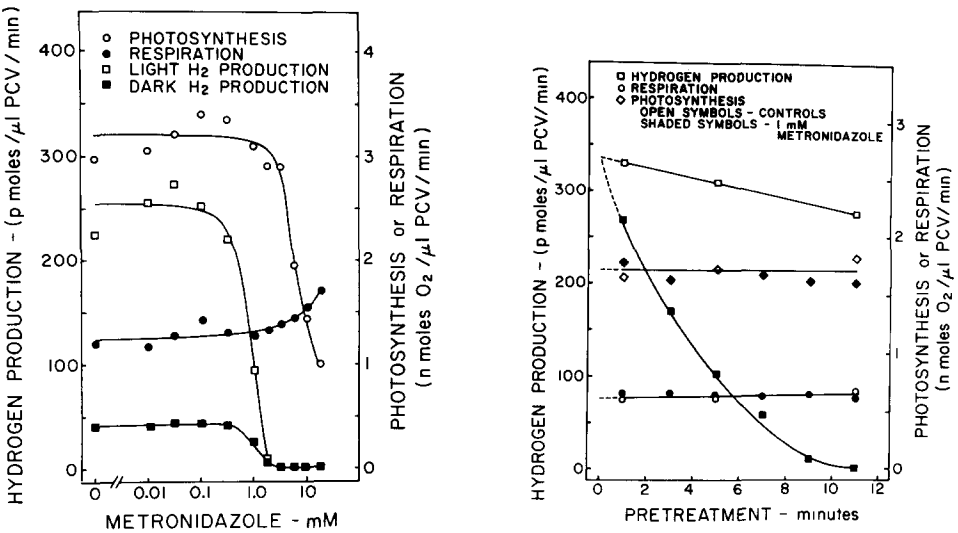


Fig. 1. Differential action of metronidazole on photosynthesis, respiration, and $H_2$ production in *A. cylindrica*. Cells were grown in continuous culture at 3.1 μl/ml and were concentrated to 20.7 μl/ml in media containing 50 mM MES buffer, pH 6.50. Conc. metronidazole in dimethyl sulfoxide was added to the algae in the cuvette 5 min before the initiation of $H_2$ evolution. $O_2$-stimulated $H_2$ production was followed for 3 min after the addition of $O_2$ to an initial concentration of 125 μM. Light-stimulated $H_2$ production and $O_2$ evolution rates were established upon irradiation with tungsten light for 2—3 min. Respiration rates were determined at approx. 100 μM $O_2$. PCV, packed cell volume.

Fig. 2. Time course of 1.0 mM metronidazole inhibition of nitrogenase, photosynthesis, and respiration in *A. cylindrica*. Cells were grown in continuous culture at 3.0 μl/ml and were concentrated to 20 μl/ml in culture media containing 20 mM MES buffer, pH 6.50. Aqueous metronidazole was added to the algae in the cuvette and incubated for different periods before determining light-stimulated $H_2$ and $O_2$ production. Respiratory rates were established in the dark at 50 μM $O_2$. Control runs of 1, 5, and 11 min are included.

time-dependent inhibition of photosynthesis or respiration was observed.

Since the nitrogenase-dependent $H_2$ production was severely poisoned, it was expected that the $C_2H_2$ reduction activity would also be similarly attenuated. Therefore, cells treated with metronidazole were tested for $C_2H_2$ reduction activity (Fig. 3). Also included in this figure is the effect of metronidazole on $CO_2$ fixation. These results are very similar to those presented in Fig. 1. 2.0 mM metronidazole strongly inhibited $C_2H_2$ reduction, whether activated aerobically in the dark or light driven, whereas no effect was seen on carbon reduction. Parallel to the results observed with $O_2$ production (Fig. 1), $CO_2$ fixation is increasingly inhibited by 3—20 mM metronidazole. These data confirm the high sensitivity of nitrogenase to lower metronidazole concentrations wherein $H_2$ production or $C_2H_2$ reduction are inhibited while $O_2$ production and $CO_2$ fixation are unaffected. If metronidazole is acting at the level of PS-I or ferredoxin in photosynthesis the similar inhibition of $O_2$ production and $CO_2$ fixation suggests that it may severely retard electron transport by acting as a poor electron acceptor while excluding other oxidants. Whether or not it acts as an uncoupler at high concentrations has not been established, but at low levels ATP-dependent processes continue. Since metronidazole inhibits electron transport processes involving ferredoxin, and because of its low reduction potential,

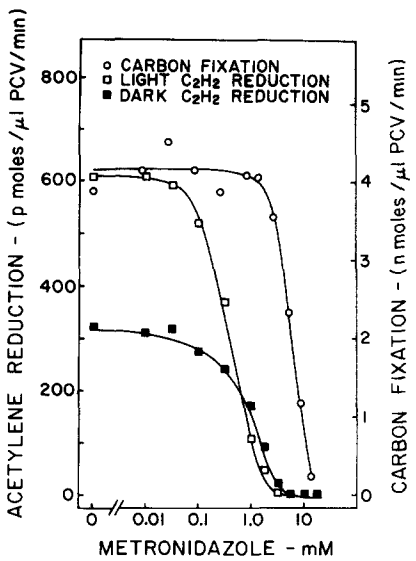


Fig. 3. Differential action of metronidazole on photosynthetic carbon fixation and $O_2$-stimulated or photostimulated $C_2H_2$ reduction. For $C_2H_2$ reduction assays cells were grown continuously at 3.0 μl/ml and were concentrated to a density of 7.5 μl/ml in culture media containing 10 mM MES buffer, pH 6.50 and inhibitor. $C_2H_2$ reduction reaction times were approx. 30 min. For $H^{14}CO_3^-$ fixation studies the cells were grown continuously at 3.2 μl/ml.

it is clear that the site of inhibition is at or near the level of ferredoxin. It is also assumed that metronidazole inhibits PS-I activity in heterocysts in a manner similar to $O_2$ production and $CO_2$ fixation in vegetative cells. Nitrogenase activity is considerably more susceptible to metronidazole poisoning, but since this activity is localized in heterocysts, it is possibly less accessible to exogenously supplied compounds. Nevertheless, these results show that the electron transport associated with $N_2$ fixation is more susceptible or accessible to metronidazole action and that metronidazole readily enters heterocysts. If the site of action is a specific ferredoxin associated with nitrogenase, it is consistent with reports that more than one form of ferredoxin occurs in blue-green algae [32—35]. Using the ferredoxin antagonist disalicylidenepropanediamine (DSPD), it was reported that photosynthesis and $C_2H_2$ reduction in *Anabaena* were inhibited to the same extent [36]. In these experiments [36] DSPD did not have the differential properties exhibited by metronidazole in our work. It has been hypothesized that a large portion and possibly all the reductant for $N_2$ fixation is provided through a dark process and not associated with photosynthetic reduction in the heterocyst [1]. The similar inhibition curves of metronidazole action on $H_2$ production and $C_2H_2$ reduction in the light and dark (Figs. 1 and 3) together with the fact that photosynthetic reactions are only inhibited at higher levels supports this hypothesis. Photosynthesis contributes to nitrogen fixation since light-fixation rates are normally greater than the best dark rates. If photosynthesis could contribute reductant to nitrogenase through direct PS-I oxidation or PS-I-associated ferredoxin, the metronidazole inhibition curve would be recognizably shifted toward higher concentrations if the inhibitor was specific for a low potential electron carrier

that is involved in dark $N_2$ fixation. This situation is not observed (Figs. 1 and 3). If, however, photosynthetic reducing ability is transferred to an electron carrier such as ferredoxin that is normally associated with nitrogenase and metronidazole is specific for this carrier, the observed results would be expected. Alternatively, metronidazole may act at the level of nitrogenase. In this case inhibition would be similar in the light and dark as we found to be the case. Therefore our data allow for several alternative or simultaneous sites for metronidazole inhibition and the possibility of light-generated reductant being used for nitrogen fixation is not excluded. The data indicate that PS-I is not inhibited at concentrations that disable the sensitive dark $N_2$ fixation reaction. Therefore, the inhibition point in $N_2$ fixation is a light-independent step common to both the dark and light $N_2$ fixation processes.

In blue-green algae $H_2$ uptake has been reported repeatedly [6,7,9,37—40]. It is also apparent that $H_2$ production may proceed concurrently with $H_2$ uptake. Since metronidazole is a strong inhibitor of nitrogenase-dependent $H_2$ production, it was of interest to see if it affected hydrogenase activity. Hydrogenase activities in *A. cylindrica* were determined and found to be low. However, the $O_2$-stimulated hydrogenase activity of *Anabaena* 7120 exceeded that of *A. cylindrica* by three to five times and the uptake hydrogenase to nitrogenase-dependent $H_2$ evolution ratio was usually greater. Consequently *Anabaena* 7120 was used to determine the effects of metronidazole on hydrogenase activity (Fig. 4). Parallel experiments were performed on *A. cylindrica* with similar results. The existence of different hydrogenase to nitrogenase ratios in various strains of *Anabaena* is in itself interesting and with little doubt accounts for the conflicting reports concerning $H_2$ production in the blue-green algae, especially with respect to $O_2$ stimulation of $H_2$ production which we can demonstrate readily in *A. cylindrica* but not at all in *Anabaena* 7120. We were also able to demonstrate the existence of two constitutive uptake hydrogenases in *Anabaena* 7120 (Fig. 4) and several other strains. The most active component in this strian is an $O_2$-dependent and cyanide-sensitive enzyme. This activity is easily distinguished from a light-stimulated $H_2$ uptake that is DCMU and cyanide insensitive. Neither of these hydrogenases was inhibited appreciably by high concentrations of $C_2H_2$ as reported for the $O_2$-dependent system in *Azotobacter* [39,41], which agrees with work on isolated heterocysts [42].

In order to measure $H_2$ uptake accurately it was desirable to eliminate the $H_2$ production activity. This was accomplished by brief pretreatments of cell suspensions with 100% $O_2$. This treatment effectively destroys the nitrogenase activity for a period sufficient to make $H_2$ uptake measurements. In cells with high hydrogenase to nitrogenase ratios, similar hydrogenase activity was detected with or without 100% $O_2$ pretreatments or with longer $O_2$ pretreatments indicating that both hydrogenases are stable to high $O_2$ concentrations. In $O_2$-poisoned cells the light-driven and the $O_2$-stimulated $H_2$ uptake proceed together and are additive at low $O_2$ concentrations in the absence of inhibitors. $O_2$ inactivation of hydrogenases observed by others [7,42] is apparently due to the cell free or disrupted systems used and probably does not represent an irreversible in vivo inactivation similar to that seen with nitrogenase.

Fig. 4 shows the effect of metronidazole on $O_2$-stimulated and light-driven $H_2$ uptake. No effect is detected on the $O_2$-dependent hydrogenase until

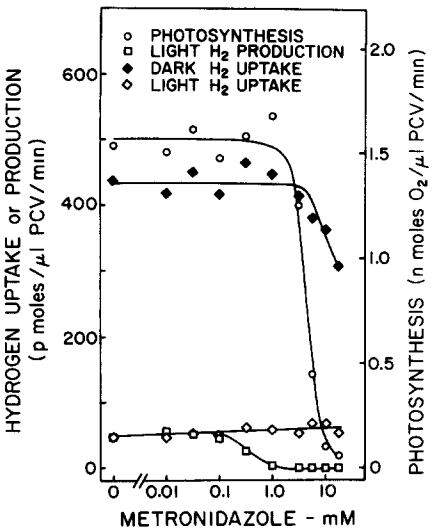


Fig. 4. Differential action of metronidazole on photosynthesis, $H_2$ production and $H_2$ uptake in *Anabaena* 7120. Cells used to determine photosynthesis and light-stimulated $H_2$ production were grown in batch culture to 4.2 μl and were concentrated to 21.0 μl/ml in 25 mM Pipes buffer, pH 6.55, with 2.5 mM $NaHCO_3$ and aqueous metronidazole. The cells were pretreated for 7 min before addition to the reaction cuvette. $H_2$ and $O_2$ production was initiated with light from a quartz-iodide source after a pretreatment time of 10 min. Cells used to determine light or $O_2$-stimulated $H_2$ uptake were obtained from batch culture of 4.5 μl/ml and were concentrated to 18 μl/ml in 22.5 mM Pipes buffer, pH 6.55, 2.25 mM $NaHCO_3$, 7.5 μM DCMU, and aqueous metronidazole. Before adding metronidazole the nitrogenase was inactivated. Cells were pretreated with metronidazole for approx. 8 min and placed under 3% $H_2$, 20% $O_2$ and argon. Approx. 10 min after introducing metronidazole the samples were added to the reaction cuvette. $O_2$-stimulated $H_2$ uptake was measured at approx. 15 μM $H_2$ and 200 μM $O_2$ in the dark, whereas light-stimulated $H_2$ uptake was measured at 7.0 μM $H_2$, and 80 μM $O_2$ after the addition of KCN to 300 μM.

3.0 mM levels are reached and approx. 30% inhibition is observed at 20 mM metronidazole. Light-stimulated $H_2$ uptake activity is not inhibited at these higher levels and may be slightly stimulated. Since photohydrogen uptake is not inhibited by DCMU, it is assumed to be a PS-I-dependent system and similar to photoreduction observed in photosynthetic bacteria or green algae. These results suggest that it is most likely that these two hydrogenases are not linked closely to ferredoxin or other low potential electron transfer components. Nevertheless, photohydrogen uptake is not inhibited by metronidazole as might be expected of a PS-I-dependent reaction. It remains possible that metronidazole acts as an electron acceptor for $H_2$ since the relative electron flux is small and metronidazole slightly stimulates photohydrogen uptake. However, this interpretation does not agree with the results obtained with photoreduction in *S. obliquus* (Fig. 6). The effects of metronidazole on nitrogenase-dependent photohydrogen production and photosynthesis are included in Fig. 4 and are nearly identical to those obtained using *A. cylindrica* (Fig. 1). Fig. 4 indicates that metronidazole inhibition remains restricted to nitrogenase activity at low concentrations and may be used as a specific poison without inhibiting these hydrogenase activities. Additionally, the same rates of $O_2$-stimulated $H_2$ uptake are observed in cells that have been pretreated 10 min with 100% $O_2$ or 1.0 mM metronidazole or with both. This finding is further

evidence that the hydrogenase activity in vivo is stable to concentrations of $O_2$ that inhibit nitrogenase.

The identities of the hydrogenases in *Scenedesmus* associated with photohydrogen production, photoreduction of $CO_2$ with $H_2$, or the oxy-hydrogen reactions are not well established although multiple forms have been detected [43]. In contrast to the hydrogenases described here for *Anabaena*, the hydrogenases of the green algae are extremely $O_2$ labile in vivo. Fig. 5 illustrates strong metronidazole inhibition of photosynthesis and a slight stimulation of respiration in *Scenedesmus* as in *Anabaena*. The sensitivity of $O_2$ production in *Scenedesmus* appears to be somewhat greater than in *Anabaena* since an effect is seen at 1.0 mM levels. Fig. 5 also shows that metronidazole has no effect on the quinone-Hill reaction. This is consistent with the action of metronidazole on low reduction potential components and indicates that PS-II is not affected. Previously it has been shown that metronidazole interacts with PS-I in *Chlamydomonas* cells and higher plant chloroplasts without inhibiting PS-II [20,23, 27].

Since photoreduction and photohydrogen production of green algae require PS-I, it would be expected that both processes would be inhibited by metronidazole in a manner similar to $O_2$ production (Fig. 5). Fig. 6 shows that this is the case for photoreduction but the inhibition of photohydrogen production is less severe. This apparent lower sensitivity of photohydrogen production is difficult to interpret as is the insensitivity of photohydrogen uptake in *Anabaena*. At 10 mM metronidazole, approx. 90% of the photoreduction is eliminated whereas about 60% of the photohydrogen production remains. It appears that the hydrogenase in photohydrogen production may associate with

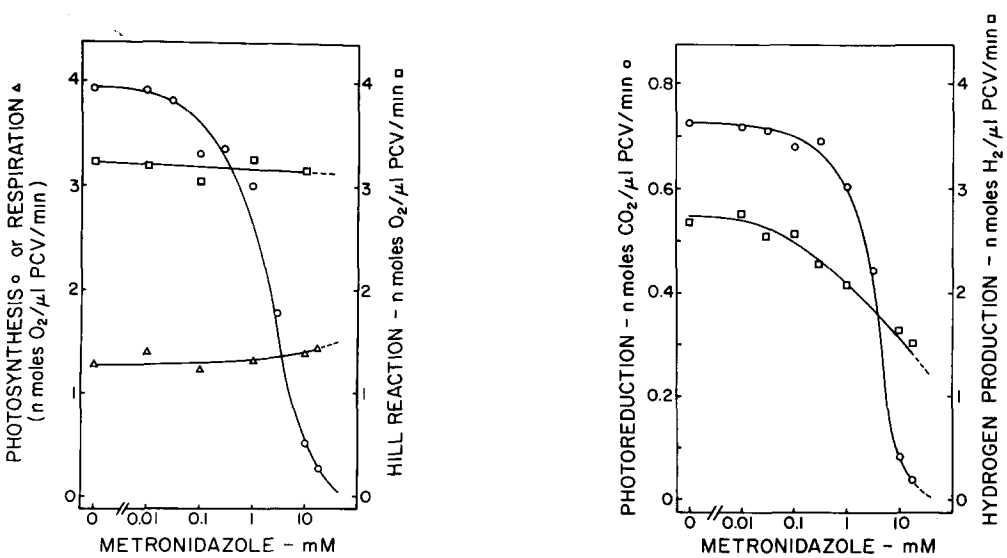


Fig. 5. Differential effects of metronidazole on photosynthetic $O_2$ production, quinone-Hill reaction, and respiration in *S. obliquus*, strain $D_3$.

Fig. 6. The effect of metronidazole on photoreduction and photohydrogen production in *S. obliquus*, strain $D_3$.

PS-I in such a manner that it can compete favorably with metronidazole as an electron acceptor or may be less susceptible to the possible production of toxic biproducts of metronidazole reduction. In *Scenedesmus* the differential inhibition of these two hydrogenase-linked reactions both involving PS-I suggests that metronidazole interferes predominantly at a sensitive site far enough from the reducing side of PS-I to strongly affect $CO_2$ reduction with less effects on the photohydrogen production pathway. The insensitivity of photohydrogen production to metronidazole is also consistent with evidence of residual photohydrogen production associated with PS-II in *P*-700 mutants of *Scenedesmus* [12].

It follows from the previous discussion of metronidazole effects on blue-green algae that if nitrogenase is directly reduced by PS-I, this interaction is more susceptible to inhibition than known pyridine nucleotide-linked PS-I reactions which involve a ferredoxin intermediate. Just the opposite appears to occur with the hydrogenase associated with PS-I in *Scenedesmus*. However, because of the dissimilarity of the two $H_2$-evolving systems, this contradiction stands as poor evidence against the direct interaction of *Anabaena* PS-I with nitrogenase.

In the preceding discussion we have shown that metronidazole is useful as a differential inhibitor in whole cells of low reduction potential reactions associated with ferredoxin. It may be found to be a useful tool to inhibit nitrogenase activity specifically in other organisms or in vitro systems. It should prove to be useful in separating certain hydrogenase activities from other low potential redox reactions in other complex systems. We have found it particularly useful in separating nitrogenase and hydrogenase activities in blue-green algae. Other applications are implicit in our discussion.

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