Low Redox Potential Promotes Sulphide- and Light-dependent Hydrogen Evolution in *Oscillatoria limnetica*

By SHIMSHON BELKIN* AND ETANA PADAN

Division of Microbial and Molecular Ecology, Institute of Life Sciences, Hebrew University, Givat Ram, Jerusalem 91904, Israel

(Received 19 April 1983; revised 25 May 1983)

Anoxygenic photosynthetic electron transport from sulphide, culminating in either H₂ evolution or CO₂ photoassimilation, was shown to include the segment from plastoquinone to ferredoxin in the cyanobacterium *Oscillatoria limnetica*. Both sulphide-dependent H₂ evolution and CO₂ photoassimilation were inhibited by plastoquinone analogues. In the former reaction, the block was bypassed by reduced *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). The link between this segment of electron transport and the hydrogenase enzyme was shown to limit the rate of sulphide-dependent H₂ evolution. The rate of flow of electrons through this pathway was lower than would be expected either from the amounts of available enzyme, as measured by the *in vitro* oxidation of reduced methyl viologen, or from rates of electron transport to CO₂ photoassimilation. When the strong reductant sodium dithionite was added to intact cells, the resulting low redox potential significantly improved photosynthetic sulphide-dependent H₂ evolution. Hydrogenase activity in cell free extracts was similarly affected by dithionite. It is suggested that ambient redox potential controls electron flow through the hydrogenase, so that surplus reducing power is removed via H₂ evolution.

INTRODUCTION

Light-dependent utilization of sulphide electrons by the cyanobacterium *Oscillatoria limnetica* requires a preliminary induction period in the presence of sulphide (Cohen *et al.*, 1975*a*; Belkin & Padan, 1978*a, b*). Low redox potential shortens the length of this adaptation. In the presence of dithionite (10 mM) the lag at pH 6.8 is eliminated (Belkin & Padan, 1983). Once adaptation is complete and sulphide electrons enter the photosynthetic electron transport chain, they can be channelled to three different acceptors: (a) CO₂, when a normal mode of anoxygenic photosynthesis takes place (Cohen *et al.*, 1975*a, b*; Oren *et al.*, 1977, 1979; Oren & Padan, 1978; Padan, 1979); (b) protons, when no CO₂ is available or when CO₂ fixation is blocked (Belkin & Padan, 1978*a, b*); (c) N₂, when no combined nitrogen is present (Belkin *et al.*, 1982).

The anoxygenic CO₂ photoassimilation reaction is unaffected by DCMU, the photosystem II inhibitor (Cohen *et al.*, 1975*a*) and inhibited by the ferredoxin-blocking agent DSPD (Belkin & Padan, 1978*b*). The sulphide-dependent H₂ evolution reaction is mediated by an hydrogenase (Belkin & Padan, 1978*a, b*) and like the former reaction it is insensitive to DCMU and inhibited by DSPD. Both systems thus share photosynthetic electron transport driven by photosystem I. This communication deals with this electron transport segment and its linkage to the hydrogenase for H₂ evolution.

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP-INT, 2,4-dinitrophenyl ether of 2-iodo-4-nitrothymol; DSPD, disalicylideneprapanediamine; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; MES, 2-(*N*-morpholino)ethanesulphonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; TAPS, tris(hydroxymethyl)methylaminopropanesulphonic acid; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

Light-driven H_2 production is basically an energy-wasteful process. However, under certain conditions, it can be utilized to rid the cells of excess reducing power, as suggested by Hillmer & Gest (1977). To investigate the influence of low redox potential on H_2 evolution in *O. limnetica*, we studied the effect of the strong reductant sodium dithionite on both intact cells and cell-free extracts. The rate-limiting step for sulphide-dependent H_2 evolution was the activity of the hydrogenase. This activity was significantly improved by the low redox potential supplied by dithionite.

METHODS

Growth of cyanobacterium. Oscillatoria limnetica (Cohen *et al.*, 1975a) was grown aerobically or anaerobically as previously described (Oren *et al.*, 1977; Oren & Padan, 1978). Exponential growth phase, in both cases, lasted 5–6 d.

Preparation of cells for experiments. Cells from exponential phase, aerobically grown cultures were harvested, washed and resuspended ($10\text{--}20\ \mu\text{g}$ chlorophyll *a* ml^{-1}) in an anaerobic growth medium lacking Na_2CO_3 and containing $10\ \mu\text{M}$ -DCMU. The medium was buffered with 25 mM-HEPES for the pH range 7.0–8.2, and 25 mM-MES or TAPS were used for lower and higher pH, respectively. Anaerobically grown cultures were similarly treated after precipitation of the sulphur deposited during anaerobic growth by a low-speed centrifugation (1000 g) for 15 s.

Cell-free extracts. Cells were washed, resuspended ($50\ \mu\text{g}$ chl. *a* ml^{-1}) in 10 mM-HEPES buffer, pH 8.0, and preincubated for 5 min in the dark at 35°C under N_2 ; lysozyme was then added to a final concentration of $1\ \text{mg}\ \text{ml}^{-1}$. Cell disruption was followed microscopically and by release of phycocyanin into the medium, as measured at A_{620} . Complete cell burst was evident after 30–45 min incubation.

H_2 evolution. Serum bottles (15 ml) containing 2 ml of intact or disrupted cell suspension were sealed with serum stoppers, flushed with N_2 for 20 min to obtain anaerobic conditions and incubated at 35°C with shaking, with or without illumination ($2.5\ \text{mJ}\ \text{cm}^{-2}\ \text{s}^{-1}$). For induction, 3.5 mM- Na_2S was added and the cells were preincubated for 3 h in the light. They were then washed and resuspended in the N_2 -flushed medium lacking Na_2S . All additions and withdrawals were made anaerobically with syringes after the N_2 flushing. H_2 was determined in gas samples as previously described (Belkin & Padan, 1978a). H_2 evolution from reduced methyl viologen was assayed in cell-free preparations in the dark, containing, in 2 ml, $25\ \mu\text{g}$ chlorophyll *a* ml^{-1} , with 2.5 mM-methyl viologen reduced by 10 mM-sodium dithionite, in 20 mM-HEPES, pH 8.0 (35°C).

Redox titrations. Determination of redox potential was carried out in an anaerobic water-jacketed (35°C) chamber, using a platinum electrode (Ingold, type 10-805-3000) with a calomel reference electrode (Radiometer, type K401). Both electrodes were connected to a pH meter (Radiometer model 26). For simultaneous pH monitoring a combined pH electrode (El-Hama, type 1160-400) was also inserted in the assay chamber and connected to an El-Hama PBS730 pH meter; samples of sodium dithionite (200 mM, in anaerobic 250 mM-MES or HEPES buffer) were added stepwise to 20 ml of the standard reaction medium which included 3.5 mM- Na_2S . Redox potential was recorded after each addition. H_2 evolution was determined in gas samples with or without illumination ($2.5\ \text{mJ}\ \text{cm}^{-2}\ \text{s}^{-1}$).

CO_2 photoassimilation. This was determined in intact *O. limnetica* cells under identical conditions to the H_2 evolution assay, but with $\text{NaH}^{14}\text{CO}_3$ injected after the N_2 flushing to a final concentration of 14 mM and a specific activity of $0.06\ \text{Ci}\ \text{mol}^{-1}$ ($2.2\ \text{GBq}\ \text{mol}^{-1}$). The radioactivity incorporated into the cells was determined as previously described (Oren *et al.*, 1977).

Chlorophyll and sulphide. There were determined according to previously described methods (Mackinney, 1941; Trüper & Schlegel, 1964).

Chemicals. Methyl viologen, HEPES, MES, TAPS, and TMPD were purchased from Sigma and $\text{NaH}^{14}\text{CO}_3$ from Amersham. DCMU and FCCP were obtained from Dupont, Wilmington, Delaware, U.S.A. DBMIB and DNP-INT were a gift from Professor M. Avron and Dr Y. Shachak of the Weizmann Institute of Science, Rehovot. $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ was from BDH and sodium dithionite from Riedle, Seelze-Hannover, F.R.G.

RESULTS

The sulphide-dependent electron transport shared by H_2 evolution and CO_2 photoassimilation

The effect of the plastoquinone antagonist DBMIB on sulphide-dependent H_2 evolution was examined. Activity was inhibited by 75–80% by $2 \times 10^{-5}\ \text{M}$ -DBMIB (Table 1), localizing the quinone in the electron transport sequence from sulphide. The electron carrier TMPD, which, when reduced, donates electrons to plastocyanin (Trebst & Reimer, 1977) and therefore bypasses the DBMIB-blocked site, restored activity to the original rate. Similar results were

Table 1. *Effect of quinone analogues on sulphide-dependent hydrogen evolution*

Two-d-old cells from an anaerobically grown *O. limnetica* culture were washed and resuspended ($14 \mu\text{g chl. } a \text{ ml}^{-1}$) in 2 ml of the anaerobic growth medium in the absence of CO_2 , and in the presence of $10 \mu\text{M-DCMU}$ and $25 \mu\text{M-HEPES}$, pH 7.5. Following a 20 min flushing with N_2 and an anaerobic addition of $3 \text{ mM-Na}_2\text{S}$, H_2 evolution was assayed in the light.

Additions (M)	H_2 evolution rate	
	$\mu\text{mol (mg chl. } a)^{-1} \text{ h}^{-1}$	%
None	12.0	100
DBMIB (2×10^{-5})	2.7	23
DBMIB (2×10^{-5}) and TMPD (10^{-4})	12.4	104
DNP-INT (2.5×10^{-5})	7.1	59
DNP-INT (2.5×10^{-5}) and TMPD (10^{-4})	10.8	90

obtained with another plastoquinone inhibitor, DNP-INT, (Trebst *et al.*, 1978) (Table 1). Although the results described in Table 1 refer to experiments conducted with anaerobically grown cells, similar results were obtained with cells induced with sulphide for only 3 h. Similar to its effect on H_2 evolution, DBMIB inhibited sulphide-dependent CO_2 photoassimilation by 80% (not shown). Both reactions were previously shown to be inhibited by the ferredoxin blocking agent DSPD (Belkin & Padan, 1978*b*). Hence it is suggested that the segment of the photosynthetic electron transport chain shared by both sulphide-dependent H_2 evolution and CO_2 photoassimilation includes the quinone, and proceeds through photosystem I up to ferredoxin.

The hydrogenase activity limits the rate of sulphide-dependent hydrogen evolution

The electrons destined for H_2 evolution exhibited much slower transport rates than those channelled to CO_2 fixation. After a 3 h induction, only $2.4 \mu\text{equiv. e}^- (\text{mg chl. } a)^{-1} \text{ h}^{-1}$ were expelled as molecular H_2 , whereas the rate of electron transfer to CO_2 was $280 \mu\text{equiv. e}^- (\text{mg chl. } a)^{-1} \text{ h}^{-1}$. Similar results were obtained with anaerobically grown cells, although in the latter system both activities markedly increased. After 3 d anaerobic growth, anoxygenic CO_2 photoassimilation rates increased twofold to $530 \mu\text{equiv. e}^- (\text{mg chl. } a)^{-1} \text{ h}^{-1}$, whereas rates of electron transfer to H_2 evolution increased more than 12-fold to $30 \mu\text{equiv. e}^- (\text{mg chl. } a)^{-1} \text{ h}^{-1}$. Even after this dramatic increase, however, nearly 95% of the electron transfer potential exhibited by the CO_2 photoassimilation reaction was not utilized for H_2 evolution. It was concluded that a rate-limiting step exists in the electron pathway from sulphide to H_2 and that this must be due to factors located beyond the common segment shared by both reactions, namely the specific electron pathway diverged to hydrogenase, and/or hydrogenase itself.

Hydrogenase potential activity can be estimated in cell-free preparations by measuring dark H_2 evolution from dithionite-reduced methyl viologen. After a 3 h induction period, the amount of enzyme present in *O. limnetica* cells maintained a rate of H_2 evolution equivalent to $18.6 \mu\text{equiv. e}^- (\text{mg chl. } a)^{-1} \text{ h}^{-1}$, which is nearly eight times higher than the light- and sulphide-dependent reaction via the electron transport chain. This pattern did not change significantly during anaerobic growth, since both the enzyme level and the rate of photosynthetic H_2 evolution increased similarly. The total hydrogenase activity was then $140 \mu\text{mol } \text{H}_2 \text{ evolved (mg chl. } a)^{-1} \text{ h}^{-1}$, which was five times higher than the photosynthetic reaction (Fig. 1). Thus the actual light-dependent expression of the enzyme comprised, at best, only 20% of the total potential activity of the enzyme. It is thus suggested that the rate-limiting factor in sulphide-dependent H_2 evolution is not the total hydrogenase level, but rather its *in vivo* activity.

Low redox potential modulates hydrogenase activity

Dithionite cannot serve as an electron donor for photosynthetic reactions in aerobically or anaerobically grown *O. limnetica* (Oren, 1978 and Fig. 2). However, when it was added to cells, induced for 3 h in the presence of sulphide, a fivefold increase from 1 to $5 \mu\text{mol } \text{H}_2 (\text{mg chl. } a)^{-1} \text{ h}^{-1}$.

Fig. 1.

Fig. 2.

Fig. 1. H₂ evolution during anaerobic growth in the absence (○) or presence (●) of 10 mM-sodium dithionite in the light at pH 8.0, and dark H₂ evolution by disrupted cells (▲) in the presence of 2.5 mM-methyl viologen reduced by 10 mM-sodium dithionite.

Fig. 2. Acceleration by sodium dithionite of sulphide-dependent H₂ evolution in 3 h induced *O. limnetica* cells. The pH was 7.3 and the following additions were made at zero time: □, none; ■, sodium dithionite (10 mM); ▲, Na₂S (3.5 mM); ●, Na₂S and sodium dithionite.

a)⁻¹ h⁻¹ occurred in light- and sulphide-dependent H₂ evolution. Although to a lesser extent, even the already enhanced rates of H₂ evolution of anaerobically grown cells were significantly increased by the addition of sodium dithionite to the reaction mixture (Fig. 1). In all cases, the light saturation behaviour of H₂ evolution did not change (not shown), and the reactions were carried out under light-saturating conditions.

The effect of dithionite on the rate of H₂ evolution was pH independent. In the presence of 10 mM of this compound, a constant maximal rate of 6 μmol H₂ (mg chl. a)⁻¹ h⁻¹ was observed throughout the pH range 6.5–9.0. When the reaction was measured in the presence of sulphide alone, however, the same high rates were attained only at pH values greater than 8.2; activity decreased to 4 μmol H₂ (mg chl. a)⁻¹ h⁻¹ at pH 8.0 and was virtually zero at pH 7.1.

Dithionite has a very low redox potential of about –450 mV (Van Dijk & Veeger, 1981), which may be the factor responsible for its effect on H₂ evolution. Sulphite, the oxidized decomposition product of dithionite, had no significant effect on the reaction. To further test the effect of low redox potential, cells were exposed to different dithionite concentrations at two pH values, and both ambient redox potentials and H₂ evolution were measured. At the two pH values tested, 6.6 (Fig. 3a) and 7.5 (Fig. 3b) the redox potentials maintained by sulphide, prior to the introduction of dithionite, were around –120 mV and –180 mV respectively, and H₂ evolution rates were minimal. These rates dramatically increased with the addition of dithionite and with the ensuing decrease in redox potential. Even though different concentrations of dithionite were needed to create identical redox potentials at the two pH values, maximal rates were observed in both cases when the redox was lowered to –420 mV. It is concluded that at least up to pH 7.5, the low ambient redox potential imposed by dithionite is indeed the factor responsible for the acceleration of H₂ evolution rates, and that a certain redox value, around –420 mV, allows maximal *in vivo* activity.

The effect of redox potential on hydrogenase activity in cell-free extracts was measured at pH 7.5, the optimum pH for the enzyme and also the cytoplasmic pH in many prokaryotes including cyanobacteria (Falkner *et al.*, 1976; Padan *et al.*, 1981). The hydrogenase-mediated H₂ evolution was found to be dependent on redox potential in a pattern very similar to that of intact cells (Fig. 4).

Fig. 3. Ambient redox potential (○) and sulphide-dependent H_2 evolution (●) at pH 6.6 (a) or pH 7.5 (b). After the N_2 -flushing, cells were incubated in the presence of 3.5 mM- Na_2S and various concentrations of sodium dithionite. H_2 evolution was measured after linear rates had been established following the lag period.

Fig. 4. Redox potential (○) and *in vitro* hydrogenase activity (●) with different concentrations of $Na_2S_2O_4$ at pH 7.5. Maximal activity (100%) was $1.2 \mu\text{mol } H_2 \text{ evolved (mg chl. a)}^{-1} \text{h}^{-1}$.

DISCUSSION

Both anoxygenic sulphide-dependent H_2 evolution and CO_2 photoassimilation were shown to be dependent on photosystem I and to share the photosynthetic electron pathway up to ferredoxin. This common chain includes the plastoquinone site, since the quinone inhibitors DBMIB and DNP-INT inhibited both reactions. Furthermore, H_2 evolution was restored in the DBMIB-inhibited system when reduced TMPD, which bypasses the block (Trebst & Reimer, 1977), was added to the reaction mixture. Phosphorylation coupled to H_2 evolution has been implicated, since the uncoupler FCCP accelerates H_2 evolution (Belkin & Padan, 1978a). This coupling site is most probably located at the quinone level, since FCCP has no enhancing effect on the reduced TMPD bypass to plastocyanin (Belkin *et al.*, 1982). These results are in accordance with recent data implying a quinone in photophosphorylation or oxidative phosphorylation (Mitchell, 1979).

Dithionite markedly accelerated the rate of sulphide- and photosystem I-dependent H_2 evolution in *O. limnetica*. It was however, incapable of replacing sulphide as an electron donor for anoxygenic photosynthetic H_2 evolution and may thus only alleviate a rate-limiting step. This could occur at three levels in the pathway of the electrons from sulphide to H_2 : the hydrogenase, the photosynthetic electron transport and the linkage between these two components. The level of the hydrogenase enzyme in these cells as measured *in vitro* was very

high, and is therefore unlikely to limit the rate of sulphide- and light-dependent electron flow to H_2 evolution *in vivo*. Whereas the enzyme could maintain a rate of H_2 evolution equivalent to 18.6 and 140 $\mu\text{equiv. e}^- (\text{mg chl. a})^{-1} \text{ h}^{-1}$ in 3 h induced or anaerobically grown cells respectively, the corresponding light-dependent activity was only 13–21%. The electron transport rate through the pathway from plastoquinone to ferredoxin, which is common to both CO_2 photoassimilation and H_2 evolution, cannot include the rate-limiting step of the latter, since the electron flow culminating in CO_2 fixation proceeds at least 18 times faster. Therefore it is concluded that the specific electron pathway to the hydrogenase, branching from the main transport chain, includes the rate-limiting step to H_2 evolution. This step is most probably the one affected by dithionite.

Simultaneous measurements of the ambient redox potential imposed by dithionite on intact cells and the *in vivo* sulphide-dependent H_2 evolution, as well as similar experiments with cell-free enzyme preparations, revealed that the lower the redox value, the higher the activity. The redox potential below which maximal H_2 evolution rates were attained was around -420 mV , similar to the midpoint potential of both the H_2/H^+ couple and ferredoxin. Assuming that dithionite reduces both the external and the cytoplasmic redox potentials, it is suggested that the *in vivo* activity of the hydrogenase is modulated by the environmental redox potential. Dithionite had no effect on the rate of H_2 evolution above pH 8.0, when even in its absence similar rates of sulphide-dependent H_2 evolution were measured. At this point the ambient redox potential maintained by sulphide was measured to be -200 mV . It is suggested that, for an unknown reason, at this pH such a potential suffices to allow optimal flow of photosynthetically driven electrons to hydrogen.

The influence of redox potential on hydrogenase activity has been described for the purified enzymes of several bacteria (Chen, 1978; Van Dijk & Veeger, 1981; Fernandez *et al.*, 1982). In most of these cases, similarly to our results, maximal activity was reached below a certain potential, around -400 to -450 mV . The importance of the redox state in the activity of ferredoxin has also been described (Carter *et al.*, 1972; Cammack & Christou, 1978) and control of photosynthetic enzymes by redox potential via the ferredoxin/thioredoxin system has been suggested (Buchanan, 1980).

Enhanced H_2 evolution under highly reducing conditions was caused, in our case, by externally supplied dithionite. We suggest, however, that this effect mimics a physiological control mechanism, with H_2 evolution acting as a valve regulating the intracellular redox environment. Such a role has been attributed to the nitrogenase-mediated H_2 production of photosynthetic bacteria (Hillmer & Gest, 1977) and to the hydrogenase-involving reactions of several heterotrophic bacteria (Adams *et al.*, 1981) and eukaryotic algae (Kessler, 1974; Hallenbeck & Benemann, 1979). The existence of such a redox-controlled 'switch' has recently been suggested in *Proteus mirabilis*, where electrons can be selectively channelled towards the reduction of either protons or other oxidants (Krab *et al.*, 1982).

Since H_2 evolution is very wasteful in terms of energy consumption, it is essential that it operates only when the system is over-reduced. Indeed, in *O. limnetica*, in the presence of ascorbate-reduced TMPD under anaerobic conditions, the rate of H_2 evolution is very low (not shown). In the presence of sulphide, under similar conditions, the rate increases concomitantly with the lowering of the redox potential to -200 mV . Addition of dithionite, yielding an ambient redox potential of -420 mV , enhances the rate to the maximum. However, the rate of H_2 evolution is always much lower than that of CO_2 photoassimilation, although both reactions share a common segment of the electron transport chain. Furthermore, in the presence of CO_2 , H_2 evolution is drastically inhibited, even in the presence of dithionite, implying that when CO_2 photoassimilation takes place electron transport is safeguarded against leakage of electrons to H_2 . Similarly, in intact algal cells, light-dependent H_2 production was observed only in the absence of CO_2 photoassimilation (Kessler, 1974).

The ancient origin of the cyanobacteria, in the early Precambrian period (Schopf, 1974), implies that in the past they had to cope with an extremely reducing environment. *Oscillatoria limnetica* is one of the cyanobacterial species that retains a large part of the metabolic

mechanism of its ancestors, namely the anoxygenic photosynthetic apparatus. The redox-controlled H_2 valve may very well be another metabolic relic of this organism's evolutionary history.

The authors wish to thank Professor H. G. Schlegel (Göttingen University), Professor M. Avron (Weizman Institute of Science, Rehovot), Professor Z. Dori (Technion, Haifa) and Professor Z. Eisenshtat (Hebrew University, Jerusalem), for most stimulating and helpful discussions. The technical assistance of B. Arieli is most appreciated. This investigation was supported by a grant from the Niedersächsisches Ministerium für Wissenschaft und Kunst, Germany.

REFERENCES

- ADAMS, M. W. W., MORTENSON, L. E. & CHEN, J. S. (1981). Hydrogenase. *Biochimica et biophysica acta* **594**, 105–176.
- BELKIN, S. & PADAN, E. (1978*a*). Sulphide-dependent hydrogen evolution in the cyanobacterium *Oscillatoria limnetica*. *FEBS Letters* **94**, 291–294.
- BELKIN, S. & PADAN, E. (1978*b*). Sulphide-dependent hydrogen evolution and CO_2 photoassimilation by the cyanobacterium *Oscillatoria limnetica*. In *Hydrogenases: Their Catalytic Activity, Structure and Function*, pp. 387–394. Edited by H. G. Schlegel & K. Schneider. Göttingen: E. Goltze, K. G.
- BELKIN, S. & PADAN, E. (1983). Na-dithionite promotes photosynthetic sulphide utilization by the cyanobacterium *Oscillatoria limnetica*. *Plant Physiology* (in the Press).
- BELKIN, S., ARIELI, B. & PADAN, E. (1982). Sulphide-dependent electron transport in *Oscillatoria limnetica*. *Israel Journal of Botany* **31**, 199–200.
- BUCHANAN, B. B. (1980). Role of light in the regulation of chloroplast enzymes. *Annual Review of Plant Physiology* **31**, 341–374.
- CAMMACK, R. & CHRISTOU, G. (1978). Characterization of iron-sulphur centers by E.P.R. spectroscopy: comparison with model systems. In *Hydrogenases: Their Catalytic Activity, Structure and Function*, pp. 45–56. Edited by H. G. Schlegel & K. Schneider. Göttingen: E. Goltze K. G.
- CARTER, C. W., JR, KRANT, J., FREER, S. T., ALDEN, R. A., SIEKER, L. C., ADMAN, E. & JENSEN, L. H. (1972). A comparison of Fe_4S_4 clusters in high potential iron protein and in ferredoxin. *Proceedings of the National Academy of Sciences of the United States of America* **69**, 3526–3529.
- CHEN, J. S. (1978). Structure and function of two hydrogenases from the dinitrogen-fixing bacterium *Clostridium pasteurianum* W5. In *Hydrogenases: Their Catalytic Activity, Structure and Function*, pp. 57–81. Edited by H. G. Schlegel & K. Schneider. Göttingen: E. Goltze K. G.
- COHEN, Y., PADAN, E. & SHILO, M. (1975*a*). Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Journal of Bacteriology* **123**, 855–861.
- COHEN, Y., YORGENSEN, B. B., PADAN, E. & SHILO, M. (1975*b*). Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Nature, London* **257**, 489–492.
- FALKNER, G., HORNER, F., WERDAN, K. & HELDT, H. W. (1976). pH changes in the cytoplasm of the blue-green alga *Anacystis nidulans* caused by light-dependent proton flux into the thylakoid space. *Plant Physiology* **58**, 717–718.
- FERNANDEZ, V. M., MUNILLA, R. & BALLESTEROS, A. (1982). Influence of redox potential on the activity of *Clostridium pasteurianum* and *Chromatium hydrogenases*. *Archives of Biochemistry and Biophysics* **215**, 129–135.
- HALLENBECK, P. C. & BENEMANN, J. R. (1979). Hydrogen from algae. In *Photosynthesis in Relation to Model Systems (Topics in Photosynthesis, Vol. 3)*, pp. 331–364. Edited by J. Barber. Amsterdam: Elsevier/North Holland Biomedical Press.
- HILLMER, P. & GEST, H. (1977). H_2 metabolism in the photosynthetic bacterium *Rhodospseudomonas capsulata*: H_2 production by growing cultures. *Journal of Bacteriology* **129**, 724–731.
- KESSLER, E. (1974). Hydrogenase, photoreduction and anaerobic growth. In *Algal Physiology and Biochemistry*, pp. 456–473. Edited by W. D. P. Stewart. London: Blackwell Scientific Publications.
- KRAB, K., OLTMAN, L. F. & STOUTHAMER, A. H. (1982). Linkage of formate hydrogenylase with anaerobic respiration in *Proteus mirabilis*. *Biochimica et biophysica acta* **679**, 51–59.
- MACKINNEY, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* **140**, 315–322.
- MITCHELL, P. (1979). Compartmentation and communication in living systems. Ligand conduction: a general catalytic principle in chemical, osmotic and chemiosmotic reaction systems. *European Journal of Biochemistry* **95**, 1–20.
- OREN, A. (1978). *Oxygenic and anoxygenic photosynthesis in Oscillatoria limnetica*. Ph.D. thesis, Hebrew University, Jerusalem.
- OREN, A. & PADAN, E. (1978). Induction of anaerobic photoautotrophic growth in the cyanobacterium *Oscillatoria limnetica*. *Journal of Bacteriology* **133**, 558–563.
- OREN, A., PADAN, E. & AVRON, M. (1977). Quantum yields of oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 2152–2156.
- OREN, A., PADAN, E. & MALKIN, S. (1979). Sulphide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplasts. *Biochimica et biophysica acta* **546**, 270–279.
- PADAN, E. (1979). Impact of facultatively anaerobic photoautotrophic metabolism on ecology of cyanobacteria (blue-green algae). In *Advances in Microbial*

- Ecology*, vol. 3, pp. 1–48. Edited by M. Alexander. New York: Plenum Press.
- PADAN, E., ZILBERSTEIN, D. & SCHULDINER, S. (1981). pH homeostasis in bacteria. *Biochimica et biophysica acta* **650**, 151–166.
- SCHOPF, J. W. (1974). Paleobiology of the Precambrian: the age of blue-green algae. In *Evolutionary Biology*, vol. 7, pp. 1–43. Edited by T. Dobzhansky, M. K. Hecht & W. C. Steere. New York: Plenum Press.
- TREBST, A. & REIMER, S. (1977). Reversal of the inhibition of photosynthetic electron flow in the chloroplasts by an internal TMPD bypass. *Photosynthetic Organelles, Special Issue of Plant and Cell Physiology*, 201–209.
- TREBST, A., WIETOSKA, H., DRUBER, W. & KNOPS, H. J. (1978). The inhibition of photosynthetic electron flow in chloroplasts by the dinitrophenylether of bromo- or iodonitrothymol. *Zeitschrift für Naturforschung* **33c**, 919–927.
- TRÜPER, H. & SCHLEGEL, H. G. (1964). Sulphur metabolism in Thiorhodaceae. I. Quantitative measurements on growing cells of *Chromatium okerii*. *Antonie van Leeuwenhoek* **30**, 225–238.
- VAN DIJK, C. & VEEGER, C. (1981). The effects of pH and redox potential on the hydrogen production activity of the hydrogenase from *Megasphaera elsdenii*. *European Journal of Biochemistry* **114**, 209–219.