Hydrostatic Pressure: A Reversible Inhibitor of Primary Photosynthetic Processes *

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Primary photosynthetic processes under pressures of up to 1300 atm were studied by means of chlorophyll fluorescence induction (Kautsky-effect) and compared to simultaneous oxygen exchange transients determined polarographically. Chlorophyll fluorescence induction was affected by increased hydrostatic pressure in three distinct ways: 1. At 400 atm loss of the first fluorescence drop (I–D transient), reflecting inhibition of PSI activity; 2. at 400–1200 atm suppression of the fluorescence peak (D–P–S transient), indicating a block at the electron donor site of PS II; 3. at 800–1200 atm flattening of the first fluorescence rise (O–I transient), suggesting a loss of excitation energy within the pigment system. Pressure effects on oxygen exchange include inhibition of transient oxygen uptake and stimulation of the initial oxygen burst, which is paralleled by loss of the first transient fluorescence drop. Inhibition of the second oxygen burst is accompanied by the elimination of the transient fluorescence peak. The first burst only decreases with pressures exceeding 800 atm, as does the initial fluorescence rise. All pressure effects on fluorescence and oxygen exchange were reversible. Hydrostatic pressure appears to be a useful multilateral inhibitor in the study of primary photosynthetic reactions.

Introduction

Temperature has long been considered an important parameter in photosynthesis studies, yet its physical-chemical analogue, hydrostatic pressure, has had a role in the work of few photosynthesis investigators. Vidaver [1–3] studied the effects of pressure on the photosynthetic responses of several marine algae and observed that steady state oxygen production and the slower transient increases are suppressed by increasing pressure, whereas, the initial rapid O_2-burst is remarkably resistant to pressures of up to 1000 atm. In these experiments, increased temperature tended to oppose pressure-induced suppression of O_2 production, as with the pressure-temperature relations of many enzymatic reactions observed *in vivo* (Johnson *et al.* [4]) and responses of other biological systems [5, 6]. Vidaver and Chandler [7] found a reversible transformation of a PSI initiated O_2 uptake transient into an O_2 evolution transient by hydrostatic pressures in the range of 500 atm. Recently Clayton and DeVault [8] reported a partially reversible decrease in quantum efficiency of both P870 oxidation and P870 fluorescence yield at 6000 atm in bacterial reaction centers, suggesting the induction of a competitive quenching process.

Rapid changes in Chl a fluorescence intensity occur within the first moments of illumination after a period of darkness (Kautsky effect) [9]. This chlorophyll fluorescence induction is a sensitive indicator of two interacting, independent light systems in photosynthesis [10–13]. Selective inhibitors can influence photosynthetic primary partial processes and induce characteristic changes in the various phases of the induction curves [14, 15].

Next to chlorophyll fluorescence induction, oxygen exchange transients are the most direct indicators of photosynthetic primary reactions. With the onset of illumination, oxygen evolution rates vary with a complex time course [16], and these transients are also influenced differently by various inhibitors of photosynthesis [7]. Transient oxygen uptake following or accompanying the rapid burst can be isolated as a negative spike after a series of preilluminating flashes or in the presence of DCMU [17, ***]. Vidaver and French [18] related a PSI-action spectrum to this transient.

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*** DCMU = 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea.

Chlorophyll fluorescence and oxygen emission rates during the induction period were correlated by Lavorel *et al.*[19] and Joliot[20]. They distinguished two phases: During an activation period fluorescence and rate of oxygen evolution increase simultaneously, followed by a phase where fluorescence and oxygen describe antiparallel courses.

We report here on our examination of photosynthetic induction in three algae; a green unicellular species, a green marine thalloid species, and a red marine species. Simultaneous recording of O_2 and fluorescence induction indicates that hydrostatic pressure has corresponding effects on both processes. The results reflect the influence of the two photochemical systems on primary photosynthetic processes. Hydrostatic pressure proves to be useful as a multilateral yet reversible inhibitor in these studies.

Materials and Methods

Scenedesmus obliquus was grown in continuous light at 25 °C in inorganic medium as described by Kessler *et al.*[21]. The green marine alga *Ulva lobata* and the red marine alga *Porphyra perforata* were collected from the Pacific coast (Bodega Bay, Calif.) and kept in sea-water at 5 °C for no longer than two days before use. Each experiment was repeated three times or more with different samples. Relative fluorescence yield as a function of time was recorded with an apparatus similar to that described by Franck *et al.*[13]. A broad band of blue incident light was obtained from a halogen lamp in combination with Balzers' Filtraflex-K3 interference filter and Corning glass filter 14 – 96 [10 mm]. Fluorescence was detected at 688 nm (halfwidth 12 nm, Balzers B-40 interference filter) with a RCA 7102 phototube. Hydrostatic pressure was applied with an apparatus similar to that described by Vidaver[2], consisting of a pressure vessel, appropriate valves, tubing, and a pump capable of generating pressures up to 1500 atm. Bifurcated fiber optics were used; one branch directed excitation light through the conical plexiglass pressure vessel window and the other conducted emitted fluorescence to the photomultiplier. Since the measured fluorescence originates mainly from the surface of the sample the problem of self-absorption is minimized. In experiments where fluorescence and oxygen-exchange were measured simultaneously a pressure O_2-electrode was used, as first described by Vidaver[1] and improved by Chandler and Vidaver[22]. Both fluorescence induction and O_2-exchange rates were recorded on a dual beam storage oscilloscope (Tektronix). Fig. 1 shows a block diagram of the experimental set up.

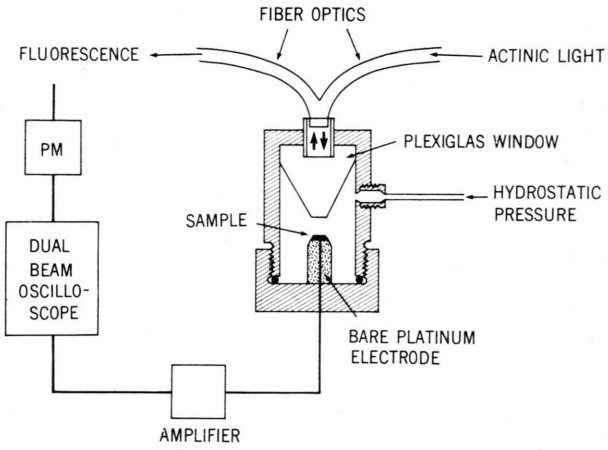

Fig. 1. Experimental set-up for simultaneous recording of fluorescence induction and oxygen exchange transients. PM = Photomultiplier.

Discs of *Ulva* or *Porphyra* thalli were held tightly against the platinum electrode surface by the means of a cellophane membrane. In case of *Scenedesmus*, the algae cells were layered onto a piece of cigarette paper which was then treated like a thallus.

Dark time before each measurement was 5 min for *Scenedesmus*, 10 min for *Ulva* and 15 min for *Porphyra* after which time the dark adapted state was reached in each of the different species. Pressure was applied for only one minute before light was given. All experiments were carried out at 25 °C.

Anaerobic conditions were achieved in the pressure vessel by operating the oxygen electrode, which consumes oxygen, for a sufficiently long time (~6 hours). Complete anaerobiosis was indicated both by a zero O_2-uptake of the electrode and an anaerobic fluorescence transient[14].

Results and Discussion

Chlorophyll fluorescence induction and hydrostatic pressure

Fig. 2 shows the effect of hydrostatic pressure on Chl a fluorescence induction in *Scenedesmus obliquus*. The various parts of the induction curve are influenced differently by increments of pressure. Most sensitive to pressure is the I – D drop (nomenclature after Munday and Govindjee[12]), which disappears at 400 atm. With higher pressures the D – P rise and the P – S drop gradually become flattened,

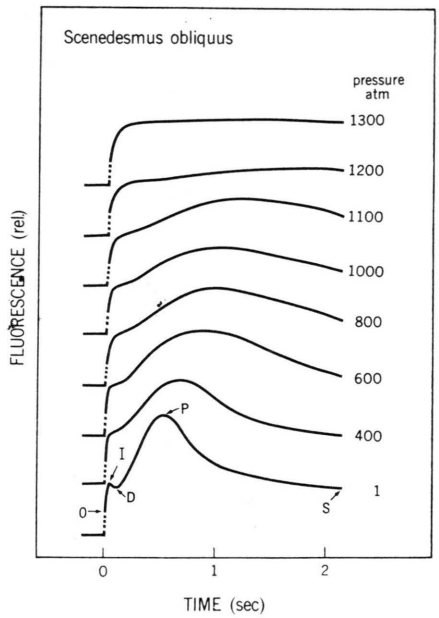

Fig. 2. Hydrostatic pressure effect on fluorescence induction in *Scenedesmus obliquus*; 10^4 erg·sec^{-1}·cm^{-2}. Notations: 0 = initial fluorescence, I = first peak, D = dip, P = peak, S = stationary fluorescence.

while the whole D – P – S transient decreases, shifting to longer times and finally disappearing at 1300 atm. There is little effect of pressure on the 0 – I rise, although it may be slightly slower at pressures exceeding 1000 atm. In the presence of DCMU the pressure effect on the 0 – I rise is more obvious, as thermal reactions are blocked by the inhibitor and the O – I rise now reflects photochemical reactions alone (Fig. 3). With DCMU there is no change from 1 – 800 atm, but at 1000 – 1200 atm the kinetics are slower and the stationary fluorescence yield is lower. With or without DCMU the effect of even the highest pressures used is reversible upon release of pressure, unless it has been applied for longer than about 5 min. Much the same responses were observed in other kinds of algae. Fig. 4 a shows pres-

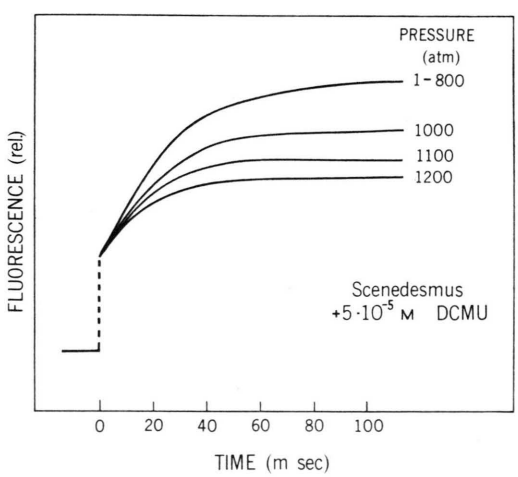

Fig. 3. Pressure effect on 0 – I fluorescence rise in the presence of DCMU; $2 \cdot 10^4$ erg·sec^{-1}·cm^{-2}.

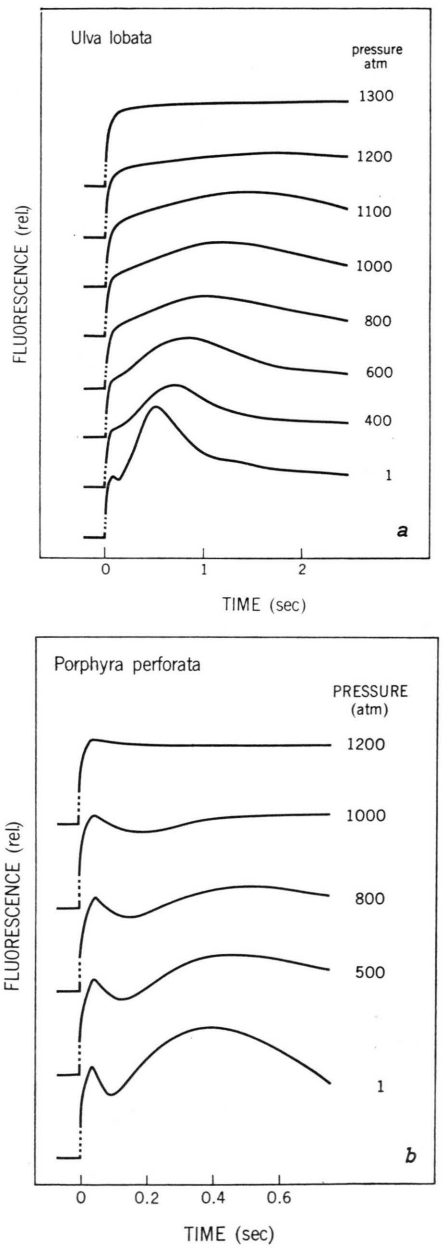

Fig. 4. Pressure effects on fluorescence induction in two marine algae.
a. The green alga *Ulva lobata*; 10^4 erg·sec^{-1}·cm^{-2}.
b. The red alga *Porphyra perforata*; $3 \cdot 10^4$ erg·sec^{-1}·cm^{-2}.

sure effects very similar to those with *Scenedesmus* on the green marine alga *Ulva lobata*. The 0 – I rise is the least affected by pressure while the D – P – S maximum is gradually eliminated and the I – D drop again disappears at 400 atm. In *Porphyra* contrastingly, the I – D drop seems to be no more sensitive to pressure than the D – P – S transient (Fig. 4 b).

All of the red algae studied showed a remarkable rise in phycobilin fluorescence induced by hydrostatic pressures exceeding 1000 atm. Fig. 5 shows

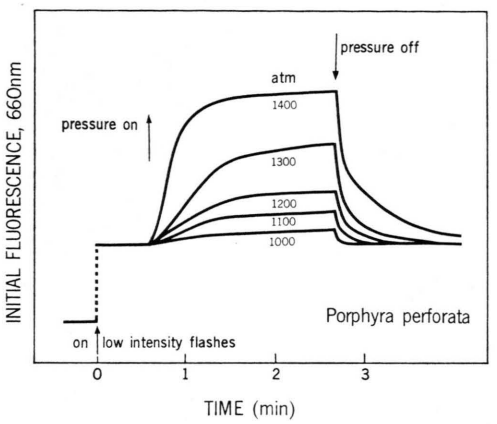

Fig. 5. Stimulation of allophycocyanin fluorescence at 660 nm by hydrostatic pressure in *Porphyra perforata*. Fluorescence was excited by 8 msec flashes of low intensity, 500 erg·sec^{-1}·cm^{-2}, to avoid possible photooxidation under pressure and contamination of the signal by variable Chl a fluorescence.

the stimulation of allophycocyanin fluorescence yield in *Porphyra perforata* by pressure and its reversibility upon pressure release. In a previous study of other aspects of this phenomenon with spectrofluorometric methods we found that pressure inhibits reversibly energy transfer from the phycobilin accessory pigments to Chl a [23]. A similar effect may occur in green algae, which could account for the lowering of the quantum yield resulting in the flattening of the 0 – I rise under DCMU (Fig. 3). However, any pressure inhibition of energy transfer in green algae is not clear, as Chl b is non-fluorescent, and preliminary studies under pressure do not show any emergent Chl b emission at 665 nm. Another explanation for the loss in quantum efficiency at high pressures could be the induction of a new quenching process by pressure, as suggested by Clayton and DeVault [8] in the case of photosynthetic bacterial reaction centers. A possible candidate for this quenching is molecular oxygen, which is known to quench Chl a fluorescence [24], and furthermore its partial pressure [activity] increases about 4-fold in an air-equilibrated, closed system with 1000 atm [25]. An obvious effect of increased oxygen activity under pressure is the disappearance of the I – D fluorescence drop at 400 atm. This transient can be isolated under anaerobic conditions in *Scenedesmus* [13–15] and then does not show any significant inhibition by pressures up to 1300 atm, as shown in Fig. 6. The high pressure sensitivity of the transient

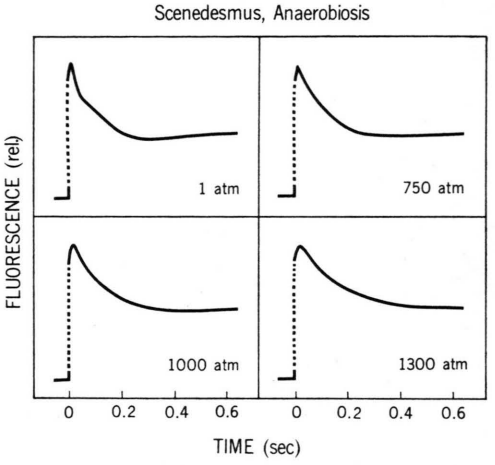

Fig. 6. Effect of pressure on the anaerobic I—D transient in *Scenedesmus obliquus*; 10^4 erg·sec^{-1}·cm^{-2}. Anaerobic conditions in the pressure vessel were achieved by operating an oxygen consuming electrode for 6 hours.

in the presence of oxygen and low sensitivity in the absence of oxygen indicate an effect of oxygen with high pressure. The I – D transient has recently been shown to display a typical PS I-action spectrum [26] and seems to reflect reoxidation of plastoquinone and the quencher Q [27] by PS I activity. The question arises of where oxygen interferes, as it suppresses the I – D drop: a. Is it at the PS I reaction centers (P700) by quenching excitation energy, or b. somewhere between the two photosystems, by oxidizing electron carriers and thus preventing P700 from being reduced [7]. Both possibilities would lead to an inhibition of PS I activity. We tend to favour possibility a., as b. should result in a light induced oxygen uptake by the algae, which has been shown to be strongly inhibited under pressure [7].

Oxygen exchange transients simultaneously recorded with chlorophyll fluorescence under pressure

When oxygen exchange rates are recorded simultaneously with chlorophyll fluorescence, the effects

of hydrostatic pressure on both induction phenomena have parallel features (Fig. 7). The disappearance of the I – D dip in fluorescence goes along with the loss of the transient O_2-uptake at 500 atm. Like the I – D dip the O_2-uptake is believed to reflect PSI activity [18] and we might conclude from

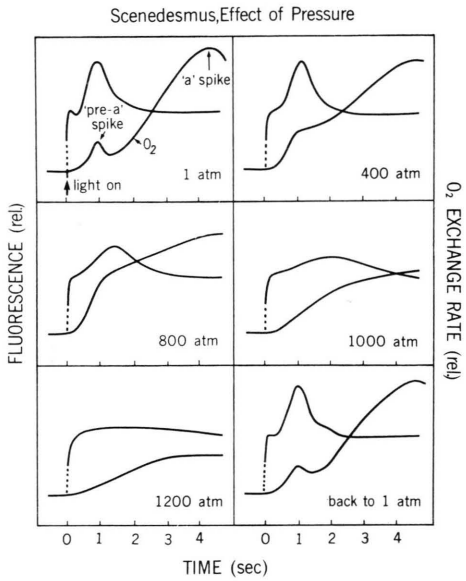

Fig. 7. Simultaneous recordings of Chl a fluorescence and oxygen exchange rate in *Scenedesmus obliquus* under pressure; 10^4 erg·sec^{-1}·cm^{-2}. The 1 atm curves after 1200 atm treatment are restored within 5 min.

the fluorescence effects that the inhibition of this uptake is caused by oxygen under high pressure. While the uptake disappears the first oxygen gush is obviously stimulated. This confirms the earlier observations by Vidaver and Chandler [7] that pressure transforms an O_2-uptake transient into an O_2-evolution transient. There appear to be two possible explanations for this phenomenon, which do not rule out each other:

1. The observed O_2 transient is the sum of simultaneous uptake and evolution components; pressure may inhibit uptake preferentially.
2. Pressure changes energy distribution between the two photosystems in favour of PSII.

There is evidence [28–30] for changes in α, the fraction of quanta absorbed by PSII, during the induction period of photosynthesis. A commonly accepted hypothesis is that after light 2 a so-called "state 2" results in which α is lower than in "state 1", after light 1. On the other hand, it is known that during the induction period there occur swelling and shrinking of the chloroplasts [31, 32], which would be expected to be affected by pressure. Thus, by applying pressure we may impose a "conformational" change on the chloroplast which increases α, shifting energy normally distributed to PSI to PSII.

The 800 atm curves in Fig. 7 show that although there is a stimulation of the pre a-spike, the following rise of the a-spike is flattened, going along with a lowering of the D – P – S peak in fluorescence. The stationary rate of oxygen production is much more sensitive to pressure than the transient burst and is totally suppressed at about 1000 atm [1–3]. This is consistent with the Z-scheme and the concept of enhancement, requiring that any oxygen production exceeding the first burst is dependent on reoxidation of reduced PSII acceptors by PSI.

At higher pressures even the pre a-spike is inhibited, while in fluorescence the D – P – S peak disappears and the 0 – I rise becomes slightly flattened. In the experiment with DCMU (Fig. 3), a loss in quantum efficiency was observed with pressure of this magnitude. The curves at 1200 atm resemble those obtained with mild heat treatment which results in irreversible loss of watersplitting [33, 34]. In contrast, however, the effect of pressure on both oxygen exchange and chlorophyll fluorescence transients is highly reversible.

Conclusions

1. In the region of 400 atm in the presence of oxygen, pressure appears to be one of the few selective and reversible inhibitors of PSI activity.
2. In the range from 800 – 1200 atm, pressure reversibly inhibits water-splitting, which may reflect the blocking of a process close to the PSII reaction centers.
3. At pressures exceeding 1000 atm there is a remarkable reversible increase of phycobilin fluorescence in red algae, indicating a blockage of energy transfer from the phycobilins to Chl a.
4. Although the complex effects of hydrostatic pressure on biological systems generally and specifically on photosynthesis are far from understood, some features of pressure influence render it a useful tool in the investigation of photosynthetic reactions.

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