PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase

Murray R. Badger, Susanne von Caemmerer, Sari Ruuska and Hiromi Nakano

Phil. Trans. R. Soc. Lond. B 2000 **355**, 1433-1446
doi: 10.1098/rstb.2000.0704

THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0704

Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase

Murray R. Badger*, Susanne von Caemmerer, Sari Ruuska and Hiromi Nakano

Molecular Plant Physiology Group, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra City, ACT 2601, Australia

Linear electron transport in chloroplasts produces a number of reduced components associated with photosystem I (PS I) that may subsequently participate in reactions that reduce O_2. The two primary reactions that have been extensively studied are: first, the direct reduction of O_2 to superoxide by reduced donors associated with PS I (the Mehler reaction), and second, the rubisco oxygenase (ribulose 1,5-bisphosphate carboxylase oxygenase EC 4.1.1.39) reaction and associated peroxisomal and mitochondrial reactions of the photorespiratory pathway. This paper reviews a number of recent and past studies with higher plants, algae and cyanobacteria that have attempted to quantify O_2 fluxes under various conditions and their contributions to a number of roles, including photon energy dissipation. In C_3 and Crassulacean acid metabolism (CAM) plants, a Mehler O_2 uptake reaction is unlikely to support a significant flow of electron transport (probably less than 10%). In addition, if it were present it would appear to scale with photosynthetic carbon oxidation cycle (PCO) and photosynthetic carbon reduction cycle (PCR) activity. This is supported by studies with antisense tobacco plants with reduced rubisco at low and high temperatures and high light, as well as studies with potatoes, grapes and madrone during water stress. The lack of significant Mehler in these plants directly argues for a strong control of Mehler reaction in the absence of ATP consumption by the PCR and PCO cycles. The difference between C_3 and C_4 plants is primarily that the level of light-dependent O_2 uptake is generally much lower in C_4 plants and is relatively insensitive to the external CO_2 concentration. Such a major difference is readily attributed to the operation of the C_4 CO_2 concentrating mechanism. Algae show a range of light-dependent O_2 uptake rates, similar to C_4 plants. As in C_4 plants, the O_2 uptake appears to be largely insensitive to CO_2, even in species that lack a CO_2 concentrating mechanism and under conditions that are clearly limiting with respect to inorganic carbon supply. A part explanation for this could be that many algal rubsicos have considerably different oxygenase kinetic properties and exhibit far less oxygenase activity in air. This would lead to the conclusion that perhaps a greater proportion of the observed O_2 uptake may be due to a Mehler reaction and less to rubisco, compared with C_3 plants. In contrast to algae and higher plants, cyanobacteria appear to have a high capacity for Mehler O_2 uptake, which appears to be not well coupled or limited by ATP consumption. It is likely that in all higher plants and algae, which have a well-developed non-photochemical quenching mechanism, non-radiative energy dissipation is the major mechanism for dissipating excess photons absorbed by the light-harvesting complexes under stressful conditions. However, for cyanobacteria, with a lack of significant non-photochemical quenching, the situation may well be different.

Keywords: Mehler reaction; oxygen photoreduction; photon energy dissipation; photorespiration; rubisco

1. INTRODUCTION

With the evolution of oxygenic photosynthesis by cyanobacteria some 2.5 billion years ago, photosynthetic organisms initiated a catastrophic change in the Earth's atmosphere and their ancestors have been coping with the developing consequences since that time. Rubisco (ribulose 1,5-bisphosphate carboxylase oxygenase EC 4.1.1.39) initially fixed CO_2 in the absence of O_2, and similarly the reduced acceptors of photosystem I (PS I) and II (PS II) reaction centres were able to transfer electrons to their intended targets without the potential intervention of O_2. A major focus of the evolution of photosynthetic organisms in a self-generated oxidative environment has been to manage the potentially damaging consequences of both these unforseen consequences and even capitalize on them where possible.

*Author for correspondence (murray.badger@anu.edu.au).

The competitive interactions between rubisco oxygenase and carboxylase have been well documented (Cleland *et al*. 1998; Roy & Andrews 2000) and it is clear that a number of strategies have been employed by various photosynthetic organisms to reduce the impact of the oxygenase reaction (Badger *et al*. 1998). Chief among these have been (i) the considerable evolutionary improvement of the kinetic properties of rubisco (Badger *et al*. 1998; Tabita 1999); (ii) the development of numerous CO_2 concentrating mechanisms (Badger *et al*. 1998; Badger & Spalding 2000); and (iii) the development of biochemical machinery to cope with phosphoglycolate, the initial product of the oxygenase reaction (Douce & Heldt 2000; Husic *et al*. 1987).

The direct photoreduction of O_2 by thylakoids has also been extensively studied. Oxygen can interact with a number of components of the photosynthetic electron transport chain, including the PS II reaction centre in its triplet $^3P^*_{680}$ state (Asada 1996), the reduced plastoquinone pool (Cleland & Grace 1999; Osmond & Grace 1995), the reduced iron sulphur (FeS) centres associated with PS I (Asada 1994) and reduced stromal acceptors such as ferredoxin (Fd) and monodehydroascorbate reductase (MDAR) (Asada 1999; Badger 1985). However, it is clear that in general the latter interactions of O_2 with PS I are the most quantitatively important in producing reactive O_2 radicals and are predominantly responsible for the direct photoreduction of O_2 by thylakoids, known as the Mehler reaction (Asada 1999; Badger 1985). Evolutionary changes associated with either suppressing the potential for direct O_2 photoreduction or coping with the reactive O_2 species produced have been only briefly dealt with by a small number of studies (Asada 1996). Most evidence points to changes in the strategies of inactivating reactive O_2 molecules, i.e. dealing with the products, while there is a scarcity of any information about changes directed at suppressing the primary photoreduction steps.

The functional activities of O_2 uptake reactions mediated by both rubisco and Mehler have been of considerable interest in interpreting various aspects of photosynthetic physiology. For rubisco, the potentially inhibitory effects of O_2 on decreasing the rates of CO_2 fixation and producing photorespiratory substrates have been uppermost (Ogren 1984). However, consideration of how electron flow supported by rubisco oxygenase at limiting CO_2 may play a role in minimizing photoinhibitory damage by excess light has also been of significant interest (Kozaki & Takeba 1996; Osmond & Björkman 1972; Osmond & Grace 1995). For Mehler O_2 photoreduction, the emphasis has been on trying to determine what rates of electron transfer to O_2 are achieved under various environmental conditions, and what reduced thylakoid and stromal components of the electron transport chain are primarily responsible for reduction of O_2 (Asada 1999; Badger 1985; Polle 1996). There has also been interest in Mehler reaction as a means of mediating additional ATP generation to meet the needs of both C_3 and C_4 photosynthesis (for a review, see Badger 1985). As for rubisco, however, considerable argument has also been expended on estimating to what extent direct photoreduction of O_2 could also lead to photon energy dissipation and protecting from photoinhibition (Osmond & Grace 1995).

This paper presents a review of recent and past data and experiments examining the quantitative roles of both the rubisco oxygenase reaction and thylakoid O_2 photoreduction (Mehler reaction) in higher plants, algae and cyanobacteria. Resulting from this analysis, questions of the potential roles of photosynthetic electron transport supported by both rubisco oxygenase (and the associated photorespiration) and Mehler reactions in dissipating photochemical energy in these various phototrophs is reassessed.

2. MECHANISMS OF PHOTOSYNTHETIC O_2 UPTAKE

The two primary processes involved in photosynthetic O_2 exchange have been previously reviewed (Badger 1985), and are dominated by

(i) those reactions associated with the direct photoreduction of O_2 (Mehler reaction) to the superoxide radical, by reduced electron transport components associated with PS I; and
(ii) those reactions linked to the photorespiratory cycle, including rubisco oxygenase in the chloroplast and glycolate oxidase and catalase–peroxidase reactions in the peroxisome.

(a) *Mehler oxygen photoreduction*

The reactions that are responsible for the direct photoreduction of O_2 can be separated into two major classes.

(i) First is the interaction of O_2 with reduced FeS–X centres associated with psaA and psaB core polypeptides of PS I (for a review, see Asada 1999) to form superoxide. Although it is possible for O_2 to be reduced by PS II and reduced plastoquinone (Cleland & Grace 1999), this appears to be much less significant compared with the PS I–FeS mediated reaction.
(ii) A second potential pathway is the interaction of O_2 with stromal components that accept electrons from PS I and are associated intimately with the complex during photosynthesis. Chief among these are reduced Fe-containing ferredoxin (Furbank & Badger 1983) and the FAD enzyme MDAR (Miyake *et al*. 1998).

In addition to the above reactions, which reduce O_2 to superoxide, there are a number of stromal and thylakoid enzymes that are involved in the degradation of superoxide to water, so that the harmful effects of active O_2 species such as superoxide and H_2O_2 can be avoided. These reactions include ascorbate peroxidase and MDAR. The integration of these stromal reactions that scavenge active oxygen species with the various O_2^--producing Mehler reactions has been described as the Mehler ascorbate peroxidase (MAP) water–water cycle. This cycle has been reviewed recently by Asada (1999) and derives its name from the fact that electrons are extracted from water by PS II, used to reduce O_2, and finally re-oxidized to water by the ascorbate peroxidase cycle.

(b) *Photorespiration*

The reactions associated with photorespiration have been extensively reviewed, including consideration of the

catalytic properties of rubisco (see Cleland *et al*. 1998; Roy & Andrews 2000), and the integrated operation of chloroplastic, peroxisomal and mitochondrial reactions associated with the processing phosophoglycolate and the recycling of carbon and nitrogen to the chloroplast (Douce & Heldt 2000; Husic *et al*. 1987). In higher plants, the O_2 consumption associated with these reactions results in a net consumption of 1.5 O_2 molecules for each rubisco oxygenase reaction that fixes O_2 and produces phosophoglycolate. The metabolism of phosophoglycolate, similar to active O_2 species, is absolutely essential for survival of the photosynthetic cell and reduces the potentially damaging effects of phosophoglycolate and the loss of carbon and nitrogen that could otherwise occur (Somerville & Ogren 1982). Photorespiratory O_2 uptake is most significant in higher plants with C_3 photosynthesis, where the passive kinetic properties of rubisco are displayed. However, it is much reduced in C_4 plants where a CO_2 concentrating mechanism is present and rubisco oxygenase is effectively suppressed (Badger 1985).

(c) *Algae and cyanobacteria*

Similar O_2 consuming reactions exist in both algae and cyanobacteria, in that they have both rubisco and reduced PS I components that are capable of reducing O_2. However some differences exist that are worth noting.

For rubisco-related reactions there are three major differences compared with higher plants. First, rubisco in many algae and cyanobacteria has different kinetic properties to higher plants, and the potential for oxygenase activity at 21% O_2 is often greatly reduced (Badger *et al*. 1998). Second, metabolism of phosophoglycolate is often short circuited, so that glycolate is either excreted to the external medium or reduced by a glycolate dehydrogenase associated with the thylakoids (Goyal & Tolbert 1996; Husic *et al*. 1987). Finally, many algae and cyanobacteria have very effective CO_2 concentrating mechanisms that suppress rubisco oxygenase (Badger & Spalding 2000; Kaplan & Reinhold 1999; Moroney & Somanchi 1999).

There are also differences associated with thylakoid-related reactions where the presence of chlororespiration reactions of the thylakoid membranes are of significant importance (Bennoun 1994). Here a terminal oxidase in the thylakoid membranes can accept electrons from the b_6f complex and O_2 is consumed probably with the production of water as in cytochrome *c* oxidase. Although the activity of chlororespiration has been found in higher plants (Casano *et al*. 2000; Roldan 1999) it is much more significant in algae and cyanobacteria (Bennoun 1994; Mi *et al*. 1992, 1995). This reaction is supposedly suppressed in the light, when oxidized PS I competes for electrons.

The scavenging of active O_2 species in the stromal environment may also be different. It is recognized that many algae and cyanobacteria actually excrete H_2O_2 and have stromal enzymes that seem especially resistant to oxidative inactivation by H_2O_2 (Takeda *et al*. 1995; Tamoi *et al*. 1998, 1999). This would suggest an active O_2 metabolism that is different to higher plants, where trace amounts of H_2O_2 have been found to dramatically inhibit the thiol-regulated enzymes of the chloroplast (Kaiser 1976, 1979).

3. FACTORS POTENTIATING PHOTOSYNTHETIC OXYGEN CONSUMPTION

In trying to understand the nature and magnitude of photosynthetic O_2 fluxes associated with various phototrophs, it is important to understand what mechanistic and environmental factors may influence the occurrence of both Mehler reaction and rubisco oxygenase.

For rubisco oxygenase–photorespiration, a number of obvious factors may influence the O_2 exchange. The levels of CO_2 and O_2 at the active site of rubisco are most important. Thus stomatal limitations in higher plants and the presence of a CO_2 concentrating mechanism such as C_4 photosynthesis will obviously have a major effect on modifying rubisco-related O_2 uptake. Additionally, changes in the kinetic properties of rubisco that would alter oxygenase activity are also important. Red algal-type rubiscos, with improved CO_2–O_2 specificity are a good example of this (Badger *et al*. 1998; Uemura *et al*. 1997), but cyanobacterial rubisco also has much reduced oxygenase activity in air (Badger *et al*. 1998). Finally, the presence of a complete photorespiratory cycle in higher plants with glycolate oxidase activity, and shuttling of redox equivalents between the chloroplast, peroxisome and mitochondria, means that the potential for O_2 uptake may be enhanced at least 50% compared with algae and cyanobacteria.

For Mehler O_2 uptake, the mechanisms that may alter the potential for O_2 reduction are less well defined. A limitation of electron acceptors, such as $NADP^+$ at PS I will cause PS I FeS centres and stromal components such as Fd and MDAR to increase their reduction levels, thus potentiating increased O_2 reduction. However, the potential of a reduced component either in the thylakoid membrane or stroma to interact with O_2 may be influenced by structural modifications that limit the access of O_2 to the reduced centres of those reduced molecules. Such a level of control of Mehler reaction has not been described but is possible. On the donor side of PS I, the state of PS I reduction is controlled by limitations of intersystem electron flow imposed by thylakoid ΔpH and the cytochrome b_6f complex (Price *et al*. 1998). Thus when CO_2 and O_2 are limiting as PS I acceptors in higher plants, PS I becomes less rather than more reduced due to a slowing in the rate of intersystem electron flow and the quantum yield of both PS I and PS II remains matched. This downregulation is due to a reduced availability of ADP (ATP consumption) and a ΔpH increase, rather than a lack of $NADP^+$. In addition to the above, the nature of the active O_2 scavenging pathways may influence the potential for O_2 exchange. The potential of algae and cyanobacteria to excrete H_2O_2 to the external medium would increase the observed O_2 uptake due to the failure of reduced O_2 to be recycled to water.

Environmental factors may obviously affect the potential of rubisco and Mehler O_2 uptake. For rubisco, factors such as water stress that close stomata and limit CO_2 will increase oxygenase. In aquatic environments, where the diffusion of CO_2 and O_2 is much reduced (Badger & Spalding 2000), inorganic carbon limitation and high O_2 stress will also be developed. Oxygenase potential will increase at elevated temperatures due to its effects on the

kinetic properties of the enzyme (Badger & Collatz 1977; Jordan & Ogren 1984).

For Mehler O_2 uptake, environmental factors that lead to a potential for thylakoid and stromal components to become more reduced, and the NADPH pool to be more oxidized, will increase the O_2 photoreduction potential. This includes high light and a lack of PS I acceptors as might occur under water stress, CO_2 limitation and low temperatures.

4. MEASURING RUBISCO OXYGENASE AND MEHLER REACTIONS *IN VIVO*

Although O_2 uptake reactions have been characterized with isolated rubisco, thylakoids and even PS I particles, it is obviously of most interest to quantify the various O_2 uptake reactions *in vivo* under relevant environmental conditions. This, however, is not a trivial feat. Measuring the various O_2 uptake reactions that occur simultaneously with the evolution of O_2 at PS II is almost impossible and approaches can, at best, be only approximations.

The fact that O_2 evolved at PS II is derived from water, while O_2 uptake is from the O_2 pool in the medium, means that $^{16}O_2$ and $^{18}O_2$ and mass spectrometry can be used to resolve the gross fluxes of O_2 evolution and O_2 uptake (Hoch & Kok 1963; Mehler & Brown 1952). While this gives a definitive answer for the absolute rate of PS II driven O_2 evolution, other less direct methods and inferences must resolve the components of gross O_2 uptake. One potential problem with this technique may be encountered in photosynthetic systems where either O_2 efflux from PS II or O_2 influx may be restricted by diffusion barriers (for a review, see Badger 1985). This could lead to different O_2 isotope ratios inside the photosynthetic compartments compared with the isotopic ratios measured externally by mass spectrometry. This could happen in C_4 plants, for example, with PS II activities operational in the bundle sheath or in other organisms with a CO_2 concentrating mechanism that restricts O_2 diffusion.

Manipulation of CO_2 and O_2 levels is a common strategy to be employed. This approach assumes that (i) rubisco oxygenase is suppressed by saturating CO_2 and that the remaining light-stimulated O_2 uptake may be ascribed to Mehler linked reactions; and (ii) that oxygenase has a relatively low affinity for O_2 compared with Mehler reactions, thus allowing Mehler to proceed more effectively at low O_2. The problems with these assumptions are that Mehler reactions may also be decreased by elevated CO_2 if the NADPH pool becomes more oxidized and that some Mehler reactions, such as those in the stroma associated with Fd and MDAR, require quite high levels of O_2 for maximum activity (Furbank & Badger 1983; Miyake *et al*. 1998).

Apart from the mass spectrometric approach, quantum yield of PS II (ϕPS II) measured by chlorophyll fluorescence (Genty *et al*. 1989) can be used to measure the flux of electrons through PS II and compared with the rate of CO_2 fixation (Cornic & Briantais 1991; Ghashghaie & Cornic 1994; Laisk & Loreto 1996). This is generally most applicable at high CO_2 or low O_2 where rubisco oxygenase is suppressed, and electron flow can be assumed to be divided between PCR cycle activity and other electron acceptors such as O_2. Although this gives a good measure of PS II electron flow, the limitations to the manipulation of CO_2 and O_2 remain as discussed above.

Finally, to investigate the potential for Mehler O_2 uptake in an *in vivo* photosynthetic system, genetic or chemical means can be employed to vary the potential of both systems. This can be done, for example, by specifically decreasing the potential of the photosynthetic carbon oxidation (PCR) cycle activity by the use of antisense RNA approaches aimed at rubisco or other PCR cycle enzymes without reducing the capacity of the thylakoid and stromal reactions associated with Mehler O_2 uptake (Hudson *et al*. 1992). The most significant potential limitation of this approach is any pleiotropic compensation in antisense trangenics that might change the potential of thylakoid-related reactions. A similar approach may be to target either carbon metabolism or thylakoid reactions with 'specific inhibitors' that may be introduced into intact tissue. This has been done with algae and cyanobacteria, with compounds such as glycolaldehyde and PS I artificial acceptors (Li & Canvin 1998; Miller & Canvin 1989) but has been less used in higher plants.

5. THE ACTIVITY AND PHYSIOLOGICAL FUNCTION OF PHOTOSYNTHETIC OXYGEN UPTAKE IN PHOTOTROPHS

(a) *C_3 plants*

Although both photorespiration and the Mehler reaction can be seen as unwanted reactions resulting from the presence of high O_2 in the atmosphere, both reactions have been ascribed a role in dissipating excess light energy and thus protecting against photodamage in higher plants and other oxygenic phototrophs (Osmond & Grace 1995). The metabolic functions of the photorespiratory cycle are obviously essential for the recovery of carbon and nitrogen associated with the production of glycolate (Somerville & Ogren 1982) and C_3 plants are unable to grow without it. However, recent work with transgenic tobacco with altered levels of chloroplast glutamine synthetase (Kozaki & Takeba 1996) has clearly emphasized its role in limiting photodamage at high light.

The potential photoprotective role of the Mehler reaction has been less well documented. Various experimental approaches have been used to infer that up to 30% of electron transport could proceed directly to O_2 under various conditions (Lovelock & Winter 1996; Osmond & Grace 1995). However, the data have been equivocal and some questions have remained about the quantitative contributions of both Mehler reaction and photorespiration to supporting extra electron transport under various conditions. In an attempt to resolve the quantitative contribution of both O_2 consuming reactions to sustain electron transport, the following attempts to summarize recent and past data that may lead to a clearer picture.

(i) *Transgenics with reduced rubisco*

With the development of antisense RNA approaches to altering aspects of plant metabolism, the opportunity has arisen to study the potential contributions of Mehler and photorespiratory O_2 exchange in plants where there have been manipulations of the relative capacities of thylakoid

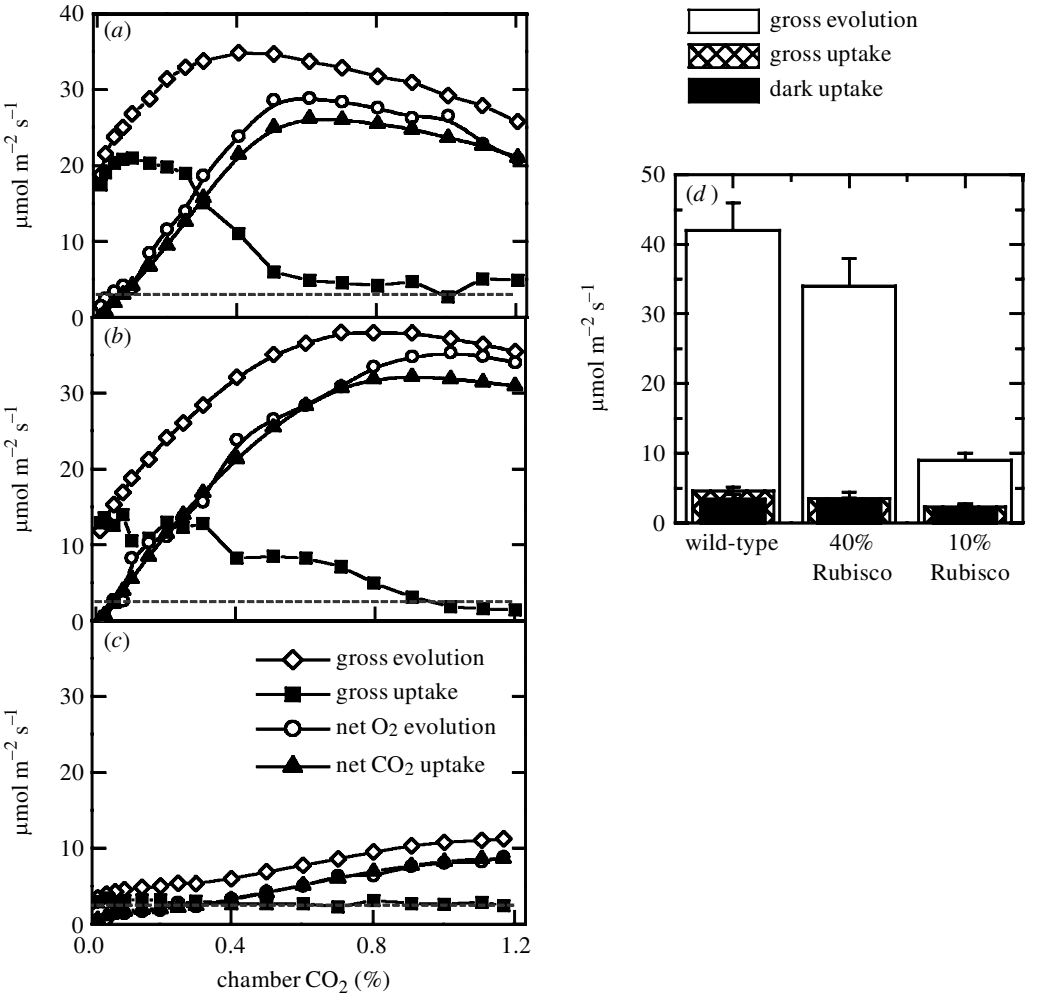

Figure 1. Net CO_2 and O_2 exchange, together with gross O_2 evolution and uptake of (*a*) wild-type and (*b*,*c*) anti-SSu tobacco ((*b*) 40% rubisco; (*c*) 10% rubisco), in response to external CO_2. The data are taken from Ruuska *et al.* (2000) and the methods are described therein. The measurements were made at 20% O_2, 970 μmol m^{-2} s^{-1} irradiance and 25 °C. (*d*) Histogram of averages of data obtained from three to four plants of each genotype. The amount of rubisco in each genotype, compared with wild-type, is shown on the graph.

electron transport and stromal rubisco, PCR and PCO cycle capacities. Recent studies of transgenic tobacco with an antisense gene directed against the mRNA of the small subunit of rubisco have provided invaluable insights into the role of O_2 as an electron acceptor during photosynthesis (Ruuska *et al.* 2000). Rubisco capacity was reduced by up to 90% in the most severely affected plants without a similar reduction in electron transport capacity, thus providing an opportunity to quantify the contribution of Mehler reaction O_2 uptake in plants where the potential contribution of photorespiratory O_2 varied greatly.

In the studies of Ruuska *et al.* (2000), concurrent measurements of chlorophyll fluorescence and CO_2 assimilation rates at different CO_2 and O_2 partial pressures showed close linear relationships between chloroplast electron transport rates calculated from chlorophyll fluorescence and from CO_2 fixation. Furthermore, these relationships were similar for wild-type and transgenic plants, indicating that the reduced capacity for rubisco carboxylase and oxygenase activity in the transgenic plants did not result in extra electron transport to some other alternative electron acceptor such as the Mehler reaction. More direct investigations of O_2 uptake reactions using mass spectrometry showed a number of results that supported this initial observation. There was an excellent correlation between electron transport rates measured from CO_2 fixation, chlorophyll fluorescence and gross O_2 evolution in wild-type and transgenics at all O_2 concentrations. In all tobacco lines studied, the dark rates of respiratory O_2 uptake were similar to the O_2 uptake in the light measured at very high CO_2, where photorespiratory O_2 uptake should be suppressed (figure 1). This strongly suggested that at high CO_2 there was little evidence for a significant light-dependent O_2 uptake such as Mehler reaction. At the CO_2 compensation point, the rates of rubisco oxygenase activity calculated from O_2 uptake were linearly related to the rubisco content of the measured leaves (figure 2). Indeed, all analyses under compensation point conditions strongly suggested that in both wild-type and transgenics light-stimulated O_2 uptake could be accounted for solely by the varying rubisco oxygenase activity in the measured plants. Thus again there was little room for inferring the operation of a significant Mehler reaction under CO_2-limited conditions.

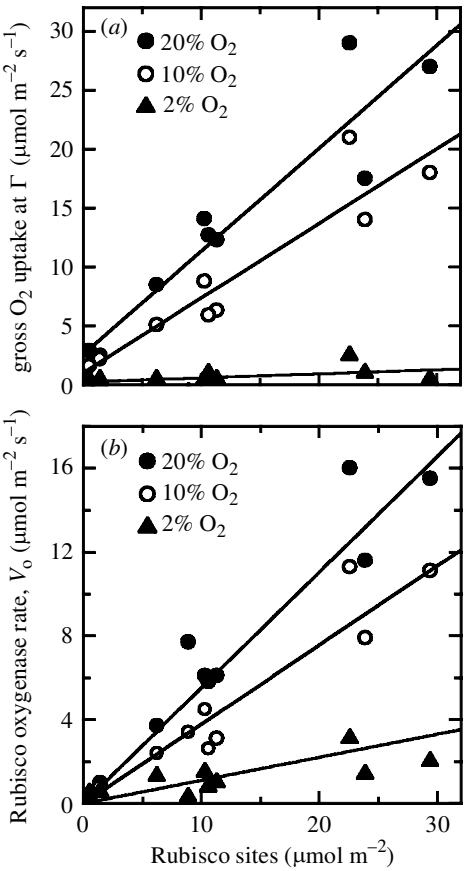

Figure 2. (*a*) Gross O_2 uptake rates and (*b*) rubisco oxygenase rates, V_o at the CO_2 compensation point, Γ. The data in (*b*) were calculated from (*a*), as a function of rubisco site concentrations in wild-type and anti-SSu tobacco plants. Measurements were made at 20%, 10% and 2% O_2 and 25 °C. The calculation of V_o was as previously described (Ruuska *et al*. 2000). The lines are the theoretical oxygenase or O_2 uptake rates predicted from the equation for oxygenase rates (for a review, see Ruuska *et al*. 2000).

(ii) *Environmental influences*

Changes in the relative contributions of both photorespiratory and Mehler O_2 uptake have been suggested to occur as a result of environmental stresses that may cause limitations to carbon assimilation or excess light interception by the photosystems (Asada 1999; Osmond & Grace 1995; Polle 1996). These stresses include high light, water deficit and both low and high temperature stresses.

(iii) *Combined water deficit and high light stress*

Closure of stomata and the presence of high light intensities generally accompany water stress in leaves. Under these conditions, plants experience their most stressful conditions with respect to a potential limitation of electron acceptors at PS I and the continued input of light energy into the chlorophyll antennae. It is therefore not surprising that there has been considerable interest in the ability of various energy dissipating mechanisms in enabling plants to minimize long-term damage under these conditions. The role of photorespiration, Mehler reaction and non-radiative (thermal) energy dissipation in the antennae have all been implicated and the quantitative contribution of each considered.

Recent studies focusing on water-stressed grapes (Flexas *et al*. 1999) and madrone (*Arbutus menziesii*) (O. Björkman and M. R. Badger, unpublished data; Osmond *et al*. 1997) have added some interesting data, pointing to a minimal role for the Mehler reaction under such conditions. One difficult aspect of studying water stress is that it is accompanied by stomatal closure that makes it difficult to achieve saturating levels of CO_2 in the chloroplast. Under these conditions, there is an over estimation of Mehler reaction at elevated CO_2 through a failure to suppress rubisco oxygenase fully. In water-stressed potatoes (Tourneux & Peltier 1994), grapes (Flexas *et al*. 1999) and madrone (O. Björkman and M. R. Badger, unpublished data) it is obvious that the CO_2 required was considerably increased compared with unstressed plants. However, when CO_2 was elevated sufficiently, sometimes requiring as high as 2% CO_2, O_2 uptake in the light was suppressed to near dark levels of O_2 uptake. In madrone at the CO_2 compensation point, high levels of O_2 uptake were observed, showing an O_2 affinity requiring in excess of 30% O_2 for half saturation. At high light intensities, the O_2 uptake rates at the compensation point in air were able to support about 50% of the maximum electron transport rate at saturating CO_2.

An analysis of the contribution of the pathways for energy dissipation in madrone under the highest irradiance and most water-stressed conditions indicated that non-radiative energy dissipation (NRD) was by far the most important, contributing in excess of 60% photon dissipation. Residual CO_2 uptake was as low as 5%, while photosynthetic O_2 uptake was responsible for the remainder (around 35%). From the O_2 exchange characteristics described above, the conclusion was reached that photorespiratory O_2 uptake probably contributed the great majority of this O_2 uptake and the Mehler reaction was only a minor component (O. Björkman and M. R. Badger, unpublished data).

However, the above conclusions are somewhat at odds with the findings of Biehler & Fock (1996), where it has been suggested that a rise in O_2 uptake during water-stress imposition in wheat is associated with a Mehler reaction. These studies attempted to measure the photorespiratory component of O_2 uptake by measuring glycolate synthesis rates. They found a decrease in glycolate production and an increased O_2 uptake attributable to Mehler reactions. However, under conditions of closing stomata, and an undefined and declining chloroplastic CO_2, it is always a strong possibility that in C_3 plants rubisco oxygenase will be stimulated.

(iv) *Temperature*

High temperatures cause a decrease in the affinity of rubisco for CO_2 while increasing the relative affinity for O_2. However, the V_{max} of both reactions increases similarly (Badger & Collatz 1977; Jordan & Ogren 1984). Thus the potential for photorespiratory O_2 uptake and energy dissipation will increase at elevated temperatures, before high temperature irreversible damage occurs to other parts of the photosynthetic machinery. However, at lower temperatures, a decrease in the activity of oxygenase activity and a decrease in V_{max} of both rubisco reactions will mean that the potential for photorespiratory O_2 uptake will decrease. When combined with high light

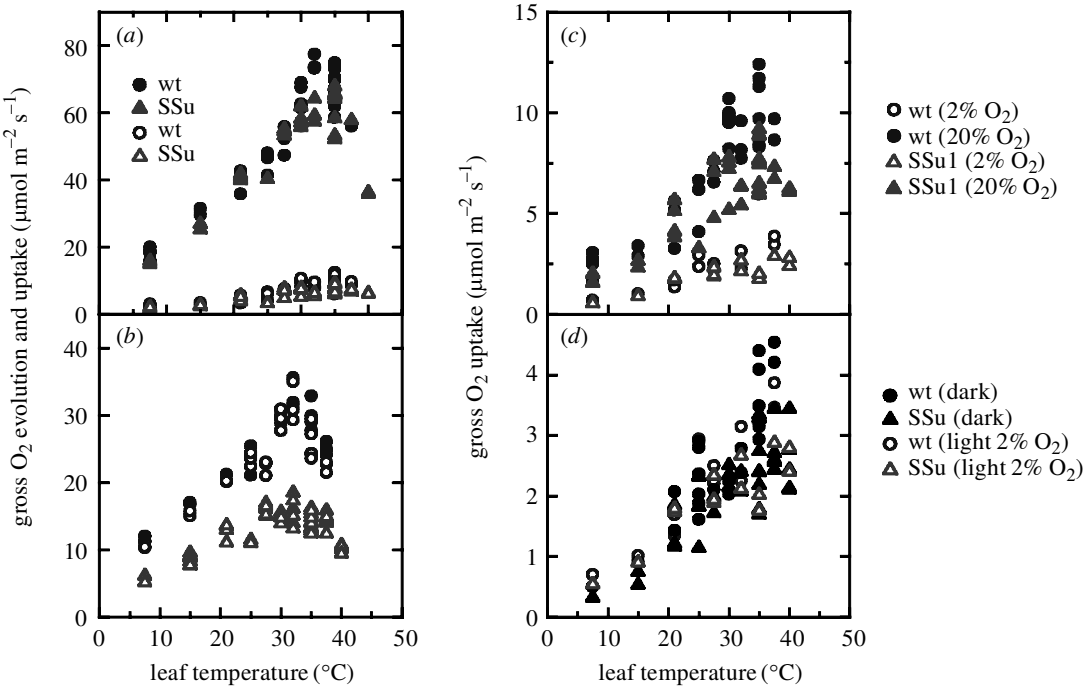

Figure 3. Gross O_2 evolution and uptake rates in wild-type and 40% anti-SSu tobacco plants as a function of leaf temperature. Fluxes at (*a*) high (2%) CO_2 and (*b*) the compensation point (Γ). (*c*,*d*) A comparison of gross O_2 uptake at high CO_2 at 2 and 21% O_2 in the light and the dark. Measurements were made at 1.7 mmol quanta $m^{-2}s^{-1}$ irradiance and various temperatures, as previously described (Ruuska *et al.* 2000). Data are from H. Nakano, S. von Caemmerer and M. R. Badger (unpublished data).

intensities, this will mean that rubisco oxygenase-supported O_2 uptake will play a greater role as an electron acceptor at elevated temperatures, and conversely will decline in importance at low temperatures. Considering this, light-stimulated Mehler reaction may be expected to increase in quantitative importance at low temperatures.

Testing this and other aspects of rubisco's response to temperature, H. Nakano, S. von Caemmerer and M. R. Badger (unpublished data) have investigated the response of photosynthetic O_2 exchange in the rubisco antisense tobacco transgenics described in §5(a)(i), with about 40% of wild-type rubisco levels. Figure 3*a* shows that over a temperature range from 7–40 °C electron transport at very high CO_2 and 21% O_2, measured by gross O_2 evolution, was similar in magnitude and response in the wild-type and transgenics. At the CO_2 compensation point, the gross O_2 evolution scaled with the rubisco content of the leaves (figure 3*b*). Considering O_2 uptake at very high CO_2 (figure 3*c*), at 2% O_2, O_2 uptake was similar in the light and in the dark in both wild-type and transgenics at all temperatures (figure 3*c*,*d*). At 21% O_2, O_2 uptake was similar between wild-type and transgenics at temperatures below 25 °C, but appeared to show a greater increase in wild-type up to 40 °C (figure 3*c*), probably indicating a inability to entirely suppress rubisco oxygenase at these higher temperatures.

The results over a wide temperature range show no evidence for any increased photosynthetic O_2 uptake at low temperatures that could be ascribed to a greater activity of Mehler reaction or any O_2 uptake at high temperatures that cannot be explained adequately by increasing rubisco oxygenase activity.

(b) *C_4 plants*

Photosynthetic O_2 uptake in C_4 plants has been previously reviewed (Badger 1985) and little new experimental evidence has been produced to change our views. The difference between C_3 and C_4 plants is primarily that the level of light-dependent O_2 uptake is generally much lower in C_4 plants and is relatively insensitive to the external CO_2 concentration. Such a major difference is readily attributed to the operation of the C_4 CO_2 concentrating mechanism that suppresses rubisco oxygenase activity in the bundle sheath. This difference, in fact, points to the conclusion that PCO cycle O_2 uptake is the major light-dependent O_2 uptake process at limiting CO_2 in C_3 plants. The phosphoenolpyruvate carboxykinase (PCK) type C_4 plants have O_2 uptakes that approach the lower end of C_3 O_2 uptake (Furbank & Badger 1982). However, this extra O_2 uptake appears to be associated with bundle sheath mitochondrial O_2 uptake, associated with NAD-malic enzyme activity involved in malate decarboxylation (Hatch 1997).

Despite the low O_2 uptake rates, particularly in NADP-malic enzyme C_4 types, photosynthetic O_2 uptake clearly has the potential to occur at quite high rates in isolated mesophyll chloroplasts of a range of C_4 species. Furthermore, the rates observed can be related to the ATP energy requirements of the substrates being metabolized (Furbank *et al.* 1983). These observations were used to infer a potential role of a Mehler reaction in C_4 mesophyll chloroplasts for the production of extra ATP via pseudocyclic photophosphorylation. Thus while the potential exists for a Mehler reaction to run in isolated chloroplasts, evidence for significant rates from intact leaf tissue is lacking. Laisk & Edwards (1998), using

chlorophyll fluorescence estimates of electron transport under various conditions in C_4 plants, have suggested that the Mehler reaction is a more important sink for electrons in C_4 plants than photorespiration; however the rates of estimated O_2 uptake were low compared with potential rates measured in isolated chloroplasts and insufficient to meet the extra ATP demands.

(c) *CAM plants*

Photosynthetic CO_2 fixation in the light in Crassulacean acid metabolism (CAM) plants can occur under two separate conditions. During the early part of the day, during phase III, photosynthesis occurs behind closed stomata while malate is being decarboxylated to release CO_2. This may be followed (phase IV) by a period when stomata open and rubisco fixes CO_2 directly from the atmosphere. Osmond & Grace (1995) noted that during phase III, Mehler O_2 uptake may represent up to 50% maximal whole-chain electron transport. This analysis would assume that rubisco was CO_2 saturated during this period, and the observed uptake may be ascribed to the Mehler reaction. Subsequent to this, Maxwell *et al.* (1998) have shown that O_2 uptake during both phase III and phase IV in *Kalenchoe diagremontiana* and *Hoya carnosa* is CO_2 sensitive and, if care is taken to ensure CO_2 saturation, there is probably little evidence for a significant light-stimulated Mehler O_2 uptake in either phase. Due to diffusional limitations imposed by both stomata and the internal leaf structure in CAM plants (Maxwell *et al.* 1997), special care needs to be taken to ensure that rubisco is CO_2 saturated and the observed light-dependent O_2 uptake cannot be due by and large to rubisco.

(i) *The role of the Mehler reaction in balancing ATP–NADPH consumption demands*

The different rates of ATP and NADPH consumption by the PCR and PCO cycles in C_3 plants and the C_4 CO_2 concentrating mechanism have led to the notion that electron transport to O_2 uptake in a Mehler reaction may serve to balance requirements and allow balanced electron transport. However, the required rate depends very much on the assumed stoichiometries. Ruuska *et al.* (2000), working with the antisense rubisco transgenic tobacco, showed that with an assumption of an H^+/ATP ratio of 3 and no Q-cycle activity, 13% of total electron flow would need to go to O_2 or other electron acceptor at high CO_2 concentrations. On the other hand an H^+/ATP ratio of 4 together with Q-cycle activity are now favoured (Haraux & de Kouchkovsky 1998; Rich 1988) and with these assumptions very little extra electron flux is actually required at high CO_2. At the CO_2 compensation point, these required flows increase to 23% and 9%, respectively. The observations that O_2 uptake at the compensation point was almost solely accounted for by rubisco oxygenase (figure 2) activity would support the latter assumption of 4 H^+ per ATP and Q-cycle activity with little involvement of Mehler activity.

(d) *Higher plant conclusions*

Based on the above, the following general conclusions may be inferred. In C_3 and CAM plants, a Mehler O_2 uptake reaction is unlikely to support a significant flow of electron transport (probably less than 10%). In addition, if Mehler reaction were present it would appear to scale with PCO and PCR cycle activity. This is supported by studies with reduced rubisco tobacco plants under both low and high temperatures and high light, as well as studies with potatoes, grapes and madrone during water stress. The lack of a significant Mehler reaction in these plants directly argues for a strong control of Mehler reaction in the absence of ATP consumption by the PCR and PCO cycles. This control is most probably exerted via an increase in ΔpH and a regulation of electron flow through the cytochrome b_6f complex. Considering this, the potential for energy dissipation at high light through Mehler reaction electron flow appears limited. Under water-stress conditions, when stomata and CO_2 may limit rubisco carboxylation, electron flow to rubisco oxygenase is probably by far the most important energy dissipative electron flow. However, it is still much less than the non-radiative photon dissipation in the light-harvesting antennae. In this respect, any small amount of Mehler electron flow may still play a role in energizing the formation of a large ΔpH, as has previously been suggested (Neubauer & Yamamoto 1992; Schreiber & Neubauer 1990).

(e) *Algae*

The potential for various types of photosynthetic O_2 exchange in algae have been previously reviewed (Badger 1985), showing a number of similarities with higher plants. However, more recent measurements on a wider range of species than previously studied (L. Franklin and M. R. Badger, unpublished data; Badger *et al.* 1998), together with a greater understanding of the evolution of the kinetic properties of rubisco, leads to the conclusion that some re-appraisal of initial conclusions may be needed.

Measurements of photosynthetic O_2 uptake and evolution in a number of non-green algal species is shown in figure 4, together with the effects of a carbonic anhydrase inhibitor, ethoxyzolamide (EZA), that decreases the effectiveness of any CO_2 concentrating mechanism. These data have been previously presented and discussed (Badger *et al.* 1998; Leggat *et al.* 1999) and serve to highlight a number of the intriguing and different aspects of photosynthetic O_2 uptake and its interpretation in algae. For three of the species, *Isochrysis* (Chrysophyta) and *Porphyridium* and *Gonniotrichopsis* (Rhodophyta), there appears to be only a small amount of light-dependent O_2 uptake when compared with C_3 higher plants, being more similar to C_4 plants in this regard. Accounting for dark respiration, the maximum light stimulated O_2 uptake represents between seven and 14% of CO_2 saturated electron transport at what would be considered high light intensities. This compares with values of 30–50% for C_3 plants (Canvin *et al.* 1980; Gerbaud & Andre 1980) and 20–30% for *Chlamydomonas reinhardtii* (Sültemeyer *et al.* 1986, 1993) and a number of other green algae (for a review, see Badger 1985). Oxygen uptake rates of around 25% have also been seen with a red macro-alga *Chondrus crispus* (Brechignac & Andre 1984, 1985) and in our laboratory with a red (*Porphyra columbina*), a brown (*Zonaria crenata*) and a green (*Ulva australis*) macro-alga (L. Franklin and M. R. Badger, unpublished data). In addition to the lower O_2

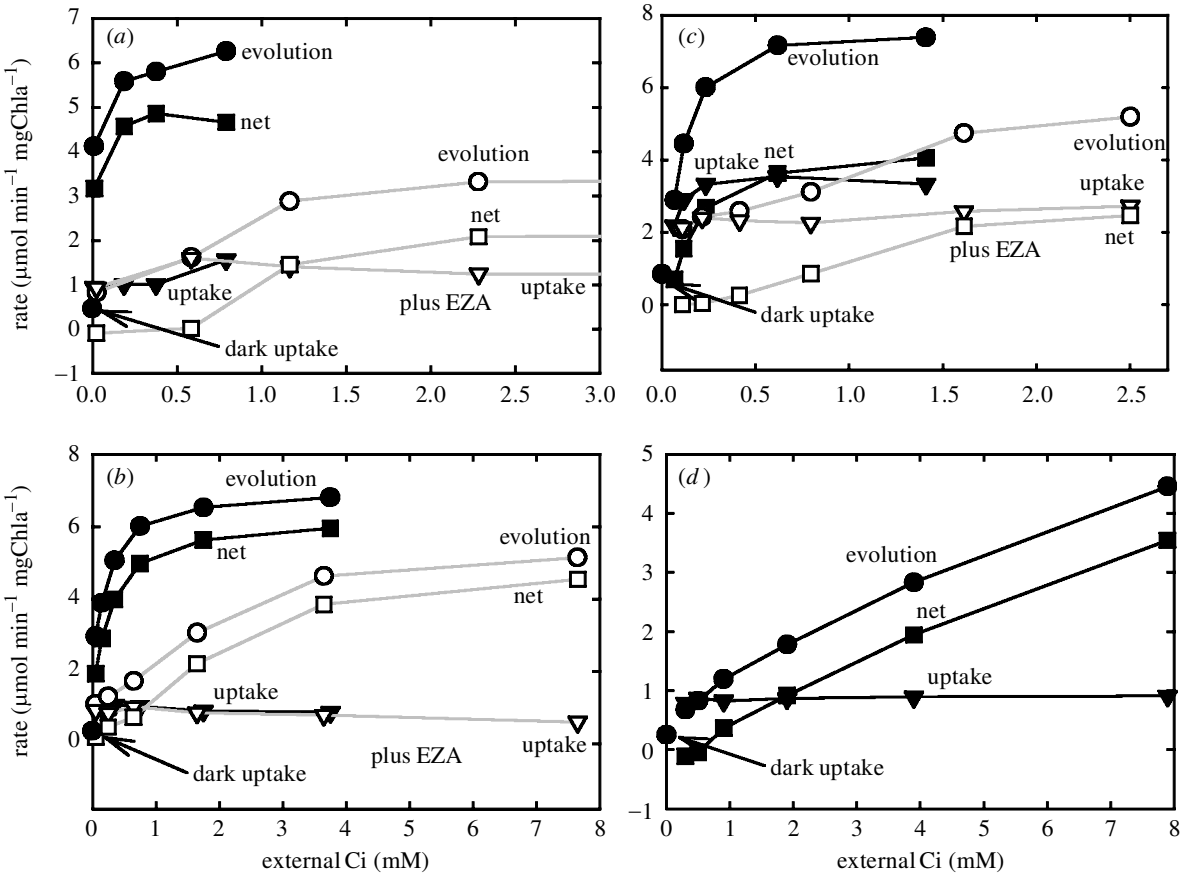

Figure 4. Photosynthesis in four species of non-green algae, (*a*) *Isochrysis galbana*, (*b*) *Porphyrium purpureum*, (*c*) *Symbiodinium* sp., (*d*) *Gonniotrichopsis sublittoralis*, in response to external Ci (inorganic carbon). The algae were grown and measured as previously described (Badger *et al*. 1998; Leggat *et al*. 1999). Measurements were at 500 μmol quanta $m^{-2} s^{-1}$. Gross O_2 evolution, gross O_2 uptake and net O_2 evolution are shown together with O_2 uptake in the dark. The carbonic anhydrase inhibitor ethoxyzolamide (EZA) was added where indicated at 500 μM.

uptake, as for C_4 plants, the uptake is relatively insensitive to CO_2 limitation, even when EZA is applied and photosynthesis is clearly limited by CO_2 availability. This apparent insensitivity is also seen in *Chondrus crispus* (Brechignac & Andre 1984; 1985) and the species studied by L. Franklin and M. R. Badger (unpublished data). In addition, the green alga *C. reinhardtii* O_2 uptake is also relatively insensitive to CO_2 (Sültemeyer *et al*. 1987), although it is stimulated considerably by increasing light intensities (Sültemeyer *et al*. 1986).

The O_2 uptake in the dinoflagellate *Symbiodinium* species is much larger and intriguing (figure 4). The maximum O_2 uptake capacity represents some 35–45% of maximum O_2 evolution and was relatively insensitive to changing CO_2 conditions and inhibition by EZA. There was even evidence for stimulation by increasing CO_2. In addition to this, the O_2 uptake observed at both high and low CO_2 appears to be saturated by as little as 10% O_2 (Leggat *et al*. 1999).

In trying to explain the photosynthetic O_2 uptake responses a number of possibilities can be raised. In many non-green algae, such as Chrysophyta, Rhodophyta and Phaeophyta, the form I rubiscos (L_8S_8—with small subunits) show considerably different kinetic properties to those of higher plants and chlorophyte algae (for a review, see Badger *et al*. 1998). These kinetics mean that at atmospheric levels of O_2, little oxygenase activity may occur (Badger *et al*. 1998). When this is combined with a CO_2 concentrating mechanism (Badger & Spalding 2000; Kaplan & Reinhold 1999) and the lack of a complete photorespiratory cycle (Husic *et al*. 1987), the net result in many algae may be low potential for light-dependent O_2 uptake and with little sensitivity to O_2. The reduced amount of O_2 uptake that is observed is probably due to some rubisco oxygenase, Mehler O_2 photoreduction and possibly chlororespiration that is much better developed in algae compared with higher plants (Bennoun 1994).

The *Symbiodinium* data need further investigation. There was an initial expectation that this alga with a form II rubisco (L_2—without small subunits) (Whitney *et al*. 1995; Whitney & Yellowlees 1995) with a potentially better developed oxygenase activity may show evidence for this in its O_2 exchange. However, the insensitivity of the uptake to decreasing CO_2 and increasing O_2 is not consistent with higher rates of photosynthetic O_2 exchange being due to rubisco oxygenase. Perhaps an explanation lies in chlororespiration and Mehler reaction.

(f) *Cyanobacteria*

Experiments with cyanobacteria over recent years have shown a number of interesting features of photosynthetic O_2 exchange that indicate some differences from algae and higher plants. Similar to non-green algae described

Table 1. *A qualitative comparison of photosynthetic O_2 uptake in phototrophs*

	phototroph			
O_2 uptake parameter	C_3	C_4	algae	cyanobacteria
O_2 uptake at high CO_2[a]	< 10%	5–15%	15–30%	20–50%
O_2 uptake at low CO_2[a]	30–50%	5–10%	15–30%	5–15%
stimulation O_2 uptake at low CO_2[b]	high	little	little	reduction
rubisco oxygenase potential at low CO_2[c]	high	low	low	low
potential Mehler versus rubisco[d]	M << R	M > R	M > R	M >> R
electron transport versus NRD for energy dissipation[e]	NRD >> ET	NRD >> ET	NRD >> ET	ET >> NRD

[a] Percentage of maximal O_2 evolution at high CO_2 and ambient O_2.
[b] O_2 uptake at low CO_2 relative to O_2 uptake at high CO_2.
[c] Potential to act as electron acceptor.
[d] An indication of the potential rates for Mehler (M) and rubisco (R) mediated photosynthetic O_2 uptake.
[e] Relative contribution of non-radiative energy dissipation (NRD) *versus* whole chain electron transport (ET) in dissipating photon energy incident on light-harvesting complexes.

above (§5(e)), photosynthetic O_2 exchange at high CO_2 may represent between 10 and 20% of maximum electron transport rates (Li & Canvin 1997*a*; Miller *et al*. 1988), although this may increase to in excess of 30% at high irradiances (Kana 1992; Li & Canvin 1997*c*). This uptake is relatively insensitive to CO_2, although rates may be slightly stimulated at low CO_2 (Li & Canvin 1997*a*). However, when carbon fixation is inhibited by compounds such as glycolaldehyde and iodoacetamide, some cyanobacteria show the ability to undertake high rates of electron transport to O_2 approaching those seen for saturating CO_2 conditions (Goosney & Miller 1997; Li & Canvin 1997*a*). Photosynthetic O_2 uptake under both inhibited and uninhibited conditions shows a low affinity for O_2 requiring in excess of 400 μM for half saturation (Li & Canvin 1997*c*).

An interesting feature of O_2 photoreduction, and indeed whole chain electron transport, is that in the absence of CO_2, rates of uptake and evolution are restricted to varying degrees (Badger & Schreiber 1993; Goosney & Miller 1997; Li & Canvin 1997*a*; Miller *et al*. 1988). Such restrictions are seen in the presence of any PS I acceptor and clearly implicate some role for inorganic carbon in controlling the rate of intersystem electron transport (Li & Canvin 1997*b*). How this occurs remains unclear but recent work understanding the role of the thylakoid NADPH dehydrogenase complex on catalysing active CO_2 uptake by cyanobacterial cells may provide an explanation (Kaplan & Reinhold 1999; Klughammer *et al*. 1999). Such an explanation would suppose that electron transport through the cytochrome b_6f complex was controlled not by the ΔpH of the thylakoid membrane but by stromal side interactions of the NADPH complex with CO_2. The fact that whole chain electron transport can occur at high rates in the absence of CO_2 fixation implies that electron flow is not tightly coupled to a thylakoid proton gradient, as is the case in higher plants. This has also been shown through the lack of effect of an uncoupler, FCCP (carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone), on electron transport to an artificial PS I acceptor (Badger & Schreiber 1993). Some aspects of a similar uncoupling of electron transport from ATP synthesis have been seen in the green alga *Scenedesmus* in the presence of PCO and PCR cycle inhibitors (Radmer & Kok 1976; Radmer & Ollinger 1978).

In interpreting O_2 uptake in cyanobacteria, the following can be concluded. Under normal photosynthetic conditions a limited rubisco oxygenase activity may occur due to the poor O_2 affinity of cyanobacteria oxygenase. There exists, however a strong potential for O_2 photoreduction, depending on the extent to which CO_2 can serve as the normal electron acceptor from PS I. Oxygen photoreduction therefore has the potential to increase at light intensities above those required to saturate CO_2 fixation and would be stimulated significantly at low CO_2 if it were not for the inhibitory effects of low CO_2 on the potential for whole-chain electron transport. A confounding factor in cyanobacteria is the relatively high rates of cyclic electron transport, much of which may proceed through the NDH1 complex and chlororespiration (Mi *et al*. 1994, 1995).

(g) *Algal and cyanobacterial conclusions*

Algae show a range of light-dependent O_2 uptake rates, similar to C_4 plants. However, there is some variation, as evidenced by the increased O_2 uptake observed in the dinoflagellate *Symbiodinium* (figure 4). However, our current understanding is limited by the low number of species that have actually been studied. As in C_4 plants, the O_2 uptake appears to be largely insensitive to CO_2, even in species that lack a CO_2 concentrating mechanism and under conditions that are clearly limiting with respect to inorganic carbon supply. A partial explanation for this could lie in the fact that many algal rubiscos may have considerably different oxygenase kinetic properties and exhibit far less oxygenase potential in air. This leads to the conclusion that perhaps a greater proportion of the observed O_2 uptake may be due to a Mehler reaction and less to rubisco, compared with C_3 plants.

In contrast to both algae and higher plants, cyanobacteria appear to have a high capacity for Mehler O_2 uptake, which appears not well coupled or limited by ATP consumption. However, the potential for Mehler reaction may be controlled by inorganic carbon, in that intersystem electron transport appears to be limited by the absence of inorganic carbon.

6. A CONCLUDING COMPARISON

Table 1 shows a qualitative comparison of the potential for photosynthetic O_2 uptake in higher plants, algae and cyanobacteria. Among all of these phototrophs, rubisco-supported O_2 uptake is a major alternative photosynthetic electron acceptor only in C_3 higher plants (and also CAM plants, data not shown). In C_4 plants, algae and cyanobacteria, the Mehler reaction may dominate, particularly in cyanobacteria where it has the potential to support up to 50% of whole-chain electron transport. It is likely that in all higher plants and algae, which have a well developed non-photochemical quenching mechanism (Niyogi 1999), NRD is the major mechanism for dissipating excess photons absorbed by the light-harvesting complexes under stressful conditions. However, for cyanobacteria, which lack significant non-photochemical quenching (Campbell *et al*. 1998), the situation may well be different. Under these circumstances, the high capacity for Mehler reaction may well serve an important role in the energy dissipation. Further study of these prokaryotic phototrophs is necessary to establish the extent to which this serves as a photoprotective mechanism.

REFERENCES

Asada, K. 1994 Production and action of active oxygen species in photosynthetic tissues. In *Causes of photooxidative stress and amelioration of defense systems in plants* (ed. C. H. Foyer & P. M. Mullineaux), pp. 77–104. Boca Raton, FL: CRC Press.

Asada, K. 1996 Radical production and scavenging in chloroplasts. In *Photosynthesis and the environment* (ed. N. R. Baker), pp. 123–150. Dordrecht, The Netherlands: Kluwer.

Asada, K. 1999 The water to water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *A. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 601–639.

Badger, M. R. 1985 Photosynthetic oxygen-exchange. *A. Rev. Plant Physiol.* **36**, 27–53.

Badger, M. R. & Collatz, G. J. 1977 Studies on the kinetic mechanism of RuBP carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic papameters. *Carnegie Inst. Wash. Yearbook* **76**, 355–361.

Badger, M. R. & Schreiber, U. 1993 Effects of inorganic carbon accumulation on photosynthetic oxygen reduction and cyclic electron flow in the cyanobacterium *Synechococcus* PCC7942. *Photosynth. Res.* **37**, 177–191.

Badger, M. R. & Spalding, M. H. 2000 CO_2 acquisition, concentration and fixation in cyanobacteria and algae. In *Photosynthesis: physiology and metabolism* (ed. R. C. Leegood, T. D. Sharkey & S. von Caemmerer), pp. 369–397. Dordrecht, The Netherlands: Kluwer.

Badger, M. R., Andrews, T. J., Whitney, S. M., Ludwig, M., Yellowlees, D. C., Leggat, W. & Price, G. D. 1998 The diversity and co-evolution of Rubisco, plastids, pyrenoids and chloroplast-based CO_2-concentrating mechanisms in the algae. *Can. J. Bot.* **117**, 1052–1071.

Bennoun, P. 1994 Chlororespiration revisited: mitochondrial-plastid interactions in *Chlamydomonas*. *Biochim. Biophys. Acta* **1186**, 59–66.

Biehler, K. & Fock, H. 1996 Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiol.* **112**, 265–272.

Brechignac, F. & Andre, M. 1984 Oxygen uptake and photosynthesis of the red macroalga, *Chondrus crispus*, in seawater. Effects of light and CO_2 concentration. *Plant Physiol.* **75**, 919–923.

Brechignac, F. & Andre, M. 1985 Oxygen uptake and photosynthesis of the red macroalga, *Chondrus crispus*, in seawater. Effects of oxygen concentration. *Plant Physiol.* **78**, 545–550.

Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P. & Oquist, G. 1998 Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.* **62**, 667–680.

Canvin, D. T., Berry, J. A., Badger, M. R., Fock, H. & Osmond, C. B. 1980 Oxygen exchange in leaves in the light. *Plant Physiol.* **66**, 302–307.

Casano, L. M., Zapata, J. M., Martin, M. & Sabater, B. 2000 Chlororespiration and poising of cyclic electron transport—plastoquinone as electron transporter between thylakoid NADH dehydrogenase and peroxidase. *J. Biol. Chem.* **275**, 942–948.

Cleland, R. E. & Grace, S. G. 1999 Voltametric detection of superoxide production by photosystem II. *FEBS Letts* **457**, 384–352.

Cleland, W. W., Andrews, T. J., Gutteridge, S., Hartman, F. C. & Lorimer, G. H. 1998 Mechanism of Rubisco—the carbamate as general base. *Chem. Rev.* **98**, 549–561.

Cornic, G. & Briantais, J.-M. 1991 Partitioning of electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. *Planta* **183**, 178–184.

Douce, R. & Heldt, H.-W. 2000 Photorespiration. In *Photosynthesis: physiology and metabolism* (ed. R. C. Leegood, T. D. Sharkey & S. von Caemmerer), pp. 115–136. Dordrecht, The Netherlands: Kluwer.

Flexas, J., Badger, M., Chow, W. S., Medrano, H. & Osmond, C. B. 1999 Analysis of the relative increase in photosynthetic O_2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. *Plant Physiol.* **121**, 675–684.

Furbank, R. T. & Badger, M. R. 1982 Photosynthetic oxygen-exchange in attached leaves of C_4 monocotyledons. *Aust. J. Plant Physiol.* **9**, 553–558.

Furbank, R. T. & Badger, M. R. 1983 Oxygen exchange associated with electron transport and photophosphorylation in spinach thylakoids. *Biochim. Biophys. Acta* **723**, 400–409.

Furbank, R. T., Badger, M. R. & Osmond, C. B. 1983 Photoreduction of oxygen in mesophyll chloroplasts of C_4 plants. *Plant Physiol.* **73**, 1038–1041.

Genty, B., Briantais, J.-M. & Baker, N. 1989 The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **990**, 87–92.

Gerbaud, A. & Andre, M. 1980 Effect of CO_2, O_2, and light on photosynthesis and photo-respiration in wheat. *Plant Physiol.* **66**, 1032–1036.

Ghashghaie, J. & Cornic, G. 1994 Effect of temperature on partitioning of photosynthetic electron flow between CO_2 assimilation and O_2 reduction and on the CO_2/O_2 specificity of Rubisco. *J. Plant Physiol.* **143**, 643–650.

Goosney, D. L. & Miller, A. G. 1997 High rates of O_2 photoreduction by the unicellular cyanobacterium *Synechocystis* PCC6803 as determined by the quenching of chlorophyll fluorescence. *Can. J. Bot.* **75**, 394–401.

Goyal, A. & Tolbert, N. E. 1996 Association of glycolate oxidation with photosynthetic electron transport in plant and algal chloroplasts. *Proc. Natl Acad. Sci. USA* **93**, 3319–3324.

Haraux, F. & de Kouchkovsky, Y. 1998 Energy coupling and ATP synthase. *Photosynth. Res.* **57**, 231–251.

Hatch, M. D. 1997 Resolving C_4 photosynthesis: trial, tribulations and other unpublished stories. *Aust. J. Plant Physiol.* **24**, 413–422.

Hoch, G. & Kok, B. 1963 A mass spectrometer inlet system for sampling gases dissolved in liquid phases. *Arch. Biochem. Biophys.* **101**, 160–170.

Hudson, G. S., Evans, J. R., Von Caemmerer, S., Arvidsson, Y. B. C. & Andrews, T. J. 1992 Reduction of ribulose 1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. *Plant Physiol.* **98**, 294–302.

Husic, D. W., Husic, H. D. & Tolbert, N. E. 1987 The oxidative photosynthetic carbon cycle or C2 cycle. *CRC Crit. Rev. Plant Sci.* **5**, 45–100.

Jordan, D. B. & Ogren, W. L. 1984 The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase oxygenase—dependence on ribulose bisphosphate concentration, pH and temperature. *Planta* **161**, 308–313.

Kaiser, W. 1976 The effect of hydrogen peroxide on CO_2 fixation of isolated chloroplasts. *Biochim. Biophys. Acta* **440**, 476–482.

Kaiser, W. M. 1979 Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplasts by hydrogen peroxide. *Planta* **145**, 377–382.

Kana, T. 1992 Relationship between photosynthetic oxygen cycling and carbon assimilation in *Synechococcus* WH7803 (cyanophyta). *J. Phycol.* **28**, 304–308.

Kaplan, A. & Reinhold, L. 1999 CO_2 concentrating mechanisms in photosynthetic microorganisms. *A. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 539–559.

Klughammer, B., Sultemeyer, D., Badger, M. R. & Price, G. D. 1999 The involvement of NAD(P)H dehydrogenase subunits, NdhD3 and NdhF3, in high-affinity CO_2 uptake in *Synechococcus* sp. PCC7002 gives evidence for multiple NDH-1 complexes with specific roles in cyanobacteria. *Mol. Microbiol.* **32**, 1305–1315.

Kozaki, A. & Takeba, G. 1996 Photorespiration protects C_3 plants from photooxidation. *Nature* **384**, 557–560.

Laisk, A. & Edwards, G. E. 1998 Oxygen and electron flow in C_4 photosynthesis—Mehler reaction, photorespiration and CO_2 concentration in the bundle sheath. *Planta* **205**, 632–645.

Laisk, A. & Loreto, F. 1996 Determining photosynthetic parameters from leaf CO_2 exchange and chlorophyll fluorescence—ribulose 1,5-bisphosphate carboxylase oxygenase specificity factor, dark respiration in the light, excitation distribution between photosystems, alternative electron transport rate, and mesophyll diffusion resistance. *Plant Physiol.* **110**, 903–912.

Leggat, W., Badger, M. R. & Yellowlees, D. 1999 Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinium* sp. *Plant Physiol.* **121**, 1247–1255.

Li, Q. G. & Canvin, D. T. 1997*a* Effect of the intracellular inorganic carbon pool on chlorophyll *a* fluorescence quenching and O_2 photoreduction in air-grown cells of the cyanobacterium *Synechococcus* UTEX 625. *Can. J. Bot.* **75**, 946–954.

Li, Q. L. & Canvin, D. T. 1997*b* Inorganic carbon accumulation stimulates linear electron flow to artificial electron acceptors of photosystem I in air-grown cells of the cyanobacterium *Synechococcus* UTEX625. *Plant Physiol.* **114**, 1273–1281.

Li, Q. L. & Canvin, D. T. 1997*c* Oxygen photoreduction and its effect on CO_2 accumulation and assimilation in air-grown cells of *Synechococcus* UTEX 625. *Can. J. Bot.* **75**, 274–283.

Li, Q. L. & Canvin, D. T. 1998 Energy sources for HCO_3^- and CO_2 transport in air-grown cells of *Synechococcus* UTEX625. *Plant Physiol.* **116**, 1125–1132.

Lovelock, C. & Winter, K. 1996 Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical tree species. *Planta* **198**, 580–587.

Maxwell, K., Von Caemmerer, S. & Evans, J. R. 1997 Is a low conductance to CO_2 diffusion a consequence of succulence in plants with crassulacean acid metabolism? *Aust. J. Plant Physiol.* **24**, 777–786.

Maxwell, K., Badger, M. R. & Osmond, C. B. 1998 A comparison of CO_2 and O_2 exchange patterns and the relationship with chlorophyll fluorescence during photosynthesis in C_3 and CAM plants. *Aust. J. Plant Physiol.* **25**, 45–52.

Mehler, A. H. & Brown, A. H. 1952 Studies on reactions of illuminated chloroplasts. III. Simultaneous photoproduction and consumption of oxygen studied with oxygen isotopes. *Arch. Biochem. Biophys.* **38**, 365–370.

Mi, H., Endo, T., Schreiber, U., Ogawa, T. & Asada, K. 1992 Electron donation from cyclic and respiratory flows to the photosynthetic intersystem chain is mediated by pyridine nucleotide dehydrogenase in the cyanobacterium *Synechocystis* PCC 6803. *Plant Cell Physiol.* **33**, 1233–1237.

Mi, H., Endo, T., Schreiber, U., Ogawa, T. & Asada, K. 1994 NAD(P)H dehydrogenase-dependent cyclic electron flow around photosystem I in the cyanobacterium *Synechocystis* PCC 6803: a study of dark-starved cells and spheroplasts. *Plant Cell Physiol.* **35**, 163–173.

Mi, H., Endo, T., Ogawa, T. & Asada, K. 1995 Thylakoid membrane-bound, NADPH-specific pyridine nucleotide dehydrogenase complex mediated cyclic electron transport in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* **36**, 661–668.

Miller, A. G. & Canvin, D. T. 1989 Glycoaldehyde inhibits CO_2 fixation in the cyanobacterium *Synechococcus* UTEX 625 without inhibiting the accumulation of inorganic carbon or the associated quenching of chlorophyll *a* fluorescence. *Plant Physiol.* **91**, 1044–1049.

Miller, A. G., Espie, G. S. & Canvin, D. T. 1988 Active transport of inorganic carbon increases the rate of O_2 photoreduction by the cyanobacterium *Synechococcus* UTEX 625. *Plant Physiol.* **88**, 6–9.

Miyake, C., Schreiber, U., Hormann, H., Sano, S. & Asada, K. 1998 The FAD-enzyme mondehydroascorbate radical reductase mediates photoproduction of superoxide radicals in spinach thylakoid membranes. *Plant Cell Physiol.* **39**, 821–829.

Moroney, J. V. & Somanchi, A. 1999 How do algae concentrate CO_2 to increase the efficiency of photosynthetic carbon fixation? *Plant Physiol.* **119**, 9–16.

Neubauer, C. & Yamamoto, H. Y. 1992 Mehler-peroxidase reaction mediates zeaxanthin formation and xeaxanthin-related fluorescence quenching in intact chloroplasts. *Plant Physiol.* **99**, 1354–1361.

Niyogi, K. K. 1999 Photoprotection revisited: genetic and molecular approaches. *A. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 333–359.

Ogren, W. L. 1984 Photorespiration—pathways, regulation, and modification. *A. Rev. Plant Physiol.* **35**, 415–442.

Osmond, C. B. & Björkman, O. 1972 Simultaneous measurements of oxygen effects on net photosynthesis and glycolate metabolism in C_3 and C_4 species of *Atriplex*. *Carnegie Inst. Wash. Yearbook* **71**, 141–148.

Osmond, C. B. & Grace, S. G. 1995 Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and ark reactions of photosynthesis? *J. Exp. Bot.* **46**, 1351–1362.

Osmond, C. B., Badger, M. R., Maxwell, K., Björkman, O. & Leegood, R. C. 1997 Too many photons: photorespiration, photoinhibition and photooxidation. *Trends Plant Sci.* **2**, 119–121.

Polle, A. 1996 Mehler reaction: friend or foe in photosynthesis? *Bot. Acta* **109**, 84–89.

Price, G. D., Von Caemmerer, S., Evans, J. R., Siebke, K., Anderson, J. M. & Badger, M. R. 1998 Photosynthesis is strongly reduced by antisense suppression of chloroplastic cytochrome *b*/*f* complex in transgenic tobacco. *Aust. J. Plant Physiol.* **25**, 445–452.

Radmer, R. J. & Kok, B. 1976 Photoreduction of O_2 primes and replaces CO_2 assimilation. *Plant Physiol.* **58**, 336–340.

Radmer, R. J. & Ollinger, O. 1978 Kinetics and apparent K_m of oxygen cycle under conditions of limiting carbon dioxide fixation. *Plant Physiol.* **61**, 915–917.

Rich, P. R. 1988 A critical examination of the supposed variable proton stoichiometry of the chloroplast cytochrome *b*/*f* complex. *Biochim. Biophys. Acta* **932**, 33–42.

Roldan, M. 1999 Can chlororespiration in plants help to explain the controversial phenotype of ndh mutants? *Trends Plant Sci.* **4**, 50.

Roy, H. & Andrews, T. J. 2000 Rubisco: assembly and mechanism. In *Photosynthesis: physiology and metabolism* (ed. R. C. Leegood, T. D. Sharkey & S. von Caemmerer), pp. 53–83. Dordrecht, The Netherlands: Kluwer.

Ruuska, S. A., Badger, M. R., Andrews, T. J. & Von Caemmerer, S. 2000 Photosynthetic electron sinks in transgenic tobacco with reduced amounts of rubisco: little evidence for significant Mehler reaction. *J. Exp. Bot.* **51**, 357–368.

Schreiber, U. & Neubauer, C. 1990 O_2-dependent electron flow, membrane energization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. *Photosynth. Res.* **25**, 279–293.

Somerville, C. R. & Ogren, W. L. 1982 Genetic modification of photorespiration. *Trends Biochem. Sci.* **7**, 171–174.

Sültemeyer, D. F., Klug, K. & Fock, H. P. 1986 Effect of photon fluence rate on oxygen evolution and uptake by *Chlamydomonas reinhardtii* suspensions grown in ambient and CO_2-enriched air. *Plant Physiol.* **81**, 372–375.

Sültemeyer, D., Klug, K. & Fock, H. P. 1987 Effect of dissolved inorganic carbon on oxygen evolution and uptake by *Chlamydomonas reinhardtii* suspensions adapted to ambient and CO_2-enriched air. *Photosynth. Res.* **12**, 25–33.

Sültemeyer, D., Biehler, K. & Fock, H. 1993 Evidence for the contribution of pseudocyclic photophosphorylation to the energy requirement of the mechanism for concentrating inorganic carbon in *Chlamydomonas*. *Planta* **189**, 235–242.

Tabita, F. R. 1999 Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a different perspective. *Photosynth. Res.* **60**, 1–28.

Takeda, T., Yokota, A. & Shigeoka, S. 1995 Resistance of photosynthesis to hydrogen peroxide in algae. *Plant Cell Physiol.* **36**, 1089–1095.

Tamoi, M., Murakami, A., Takeda, T. & Shigeoka, S. 1998 Acquisition of a new type of fructose 1,6-bisphosphatase with resistance to hydrogen peroxide in cyanobacteria—molecular characterization of the enzyme from *Synechocystis* PCC6803. *Biochim. Biophys. Acta* **1383**, 232–244.

Tamoi, M., Takeda, T. & Shigeoka, S. 1999 Functional analysis of fructose 1,6-bisphosphatase isozymes (*fbp-I* and *fbp-II* gene products) in cyanobacteria. *Plant Cell Physiol.* **40**, 257–261.

Tourneux, C. & Peltier, G. 1994 Effect of water deficit on photosynthetic oxygen exchange measured using $^{18}O_2$ and mass spectrometry in *Solanum tuberosum* L. leaf discs. *Planta* **195**, 570–577.

Uemura, K., Anwaruzzaman, Miyachi, S. & Yokota, A. 1997 Ribulose 1,5-bisphosphate carboxylase/oxygenase from thermophilic red algae with a strong specificity for CO_2 fixation. *Biochem. Biophys. Res. Commun.* **233**, 568–571.

Whitney, S. M. & Yellowlees, D. 1995 Preliminary investigations into the structure and activity of ribulose bisphosphate carboxylase from two photosynthetic dinoflagellates. *J. Phycol.* **31**, 138–146.

Whitney, S. M., Shaw, D. C. & Yellowlees, D. 1995 Evidence that some dinoflagellates contain a ribulose 1,5-bisphosphate carboxylase/oxygenase related to that of the α-proteobacteria. *Proc. R. Soc. Lond.* B **259**, 271–275.

Discussion

A. Laisk (*Institute of Molecular and Cell Biology, University of Tartu, Estonia*). Why did you not mention the possibility that electrons could be transported to the mitochondria by the malate dehydrogenase shuttle and be oxidized there? If this pathway was active, the flux denoted as Mehler reaction in your paper would have been even lower than presented in your graphs.

M. R. Badger. You are correct in pointing out that any light-generated reducing equivalents transported to the mitochondria and reducing oxygen via cytochrome *c* oxidase would be measured in our techniques as light-dependent oxygen uptake. I did not consider this for the sake of simplifying the situation, but certainly if it did occur to any significant extent it would reduce the level of any inferred Mehler reaction in our experiments.

J. F. Allen (*Department of Plant Cell Biology, Lund University, Sweden*). Oxygen can also have a catalytic role. Over-reduction of cyclic electron transport can be counteracted by a poising pulse of O_2. Do the analyses shown consider the vital catalytic role where the total O_2 consumption may be small, but the Mehler reaction would be indispensable for the initiation of photosynthesis?

M. R. Badger. Our analyses really only deal with considering electron flows to O_2 during steady-state photosynthesis and our techniques have a level of error where it would be difficult to resolve the presence of small O_2 uptakes of a few per cent or less of total whole-chain electron transport. We have not studied the period during the initiation of photosynthesis from a dark period because the light on causes thermal artefacts that would mask any significant oxygen uptake.

K. Asada (*Department of Biotechnology, Faculty of Engineering, Fukuyama University, Japan*). You showed that the electron flux through the water–water cycle proceeds at appreciable rates in C_4 plants, eukaryotic algae and cyanobacteria. What mediator participates in the enhanced photoreduction of oxygen in algae and cyanobacteria? When the water–water cycle operates just for the dissipation of excess photons, ATP is produced but not consumed. As Professor Heber has shown, chloroplasts can keep a constant level of ATP, i.e. chloroplasts can hydrolyse ATP to keep a constant level. This is just a comment.

M. R. Badger. I don't know what the mediator for oxygen uptake is in these non-higher plant systems. Reduced ferredoxin is a possibility, but the potential for monodehydroascorbate reductase to mediate this uptake (as shown by you in higher plants), may be more limited due to different activities of the ascorbate reduction and oxidation cycles in the chloroplasts of algae and cyanobacteria (see § 5(e,f) for discussion). As pointed out by Dr Matthijs (see next question), both algae and cyanobacteria have strong chlororespiratory activities on the thylakoids that could also play a role in light-stimulated oxygen uptake via a cytochrome oxidase pathway.

H. C. P. Matthijs (*Department of Microbiology, University of Amsterdam, The Netherlands*). I wish to address the difference in energy use, in which cyanobacteria have more electron transfer (to oxygen) than plants and algae. You attribute this to very high Mehler reaction in cyanobacteria. How would you be able to discriminate between Mehler reaction and direct PS II (transfer) to cytochrome aa_3 electron transfer?

M. R. Badger. The truth is that we cannot distinguish with our measurements, and the flow of electrons from PS II to a cytochrome oxidase pathway would be observed as light-stimulated oxygen uptake. However, one consequence of this would be that the electron flow through PS II would be in excess of PS I or else the intersystem pool would be drained of electrons. Recent measurements that we have been doing, comparing electron flow through both photosystems, clearly show that PS I flow is in considerable excess, indicating cyclic electron flow.

H. Griffiths (*Department of Agricultural and Environment Science, University of Newcastle, UK*). One possible limitation of the mass spectrometer method as you mentioned would be the possibility of (oxygen) recycling. In the closed system of the cuvette, what are the likely rates of this process as the CO_2–O_2 ratio changes, and can you determine the extent by monitoring the m/e 34 (i.e. $^{18}O^{16}O$ appearance)?

M. R. Badger. Recycling of O_2 species is only likely in compartments within the leaf that are quite isolated from the ambient air as a result of strong diffusional limitations. Even when stomata are relatively closed, it is hard to see recycling contributing more than a few per cent to the error of estimating oxygen uptake and evolution. As far as monitoring mass 34 is concerned, I don't immediately see how this would help. An increased evolution of mass 34, above what could be expected as a consequence of the natural isotopic abundance in water, could only occur if the water in the leaf became relatively enriched in 34. This could happen through diffusional restrictions placed on the evaporation of water, but I cannot immediately see how this would help us to derive a measure of O_2 recycling within the leaf.