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Photorespiration: metabolic pathways and their role in stress protection

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Photorespiration results from the oxygenase reaction catalysed by ribulose-1,5-bisphosphate carboxylase/oxygenase. In this reaction glycollate-2-phosphate is produced and subsequently metabolized in the photorespiratory pathway to form the Calvin cycle intermediate glycerate-3-phosphate. During this metabolic process, CO₂ and NH₃ are produced and ATP and reducing equivalents are consumed, thus making photorespiration a wasteful process. However, precisely because of this inefficiency, photorespiration could serve as an energy sink preventing the overreduction of the photosynthetic electron transport chain and photoinhibition, especially under stress conditions that lead to reduced rates of photosynthetic CO₂ assimilation. Furthermore, photorespiration provides metabolites for other metabolic processes, e.g. glycine for the synthesis of glutathione, which is also involved in stress protection. In this review, we describe the use of photorespiratory mutants to study the control and regulation of photorespiratory pathways. In addition, we discuss the possible role of photorespiration under stress conditions, such as drought, high salt concentrations and high light intensities encountered by alpine plants.

Keywords: drought stress; glutamate synthase; glutamine synthetase; glycine decarboxylase; hydroxypyruvate reductase; serine:glyoxylate aminotransferase

1. THE ORIGINS OF PHOTORESPIRATION

Photorespiration is a consequence of the oxygenation of ribulose-1,5-bisphosphate (RuBP) catalysed by RuBP carboxylase/oxygenase (rubisco). The ratio of the carboxylation rate (v_c) to the oxygenation rate (v_o) is dependent on the CO₂ and O₂ concentrations, the Michaelis constants for these gases (K_c and K_o) and the maximal velocities (V_c and V_o) (Farquhar et al. 1980): v_c/v_o = V_cK_o/V_oK_c ([CO₂]/[O₂]), with the term V_cK_o/V_oK_c defining the specificity factor of rubisco.

Using rubisco kinetics, it is possible to calculate the ratio of v_c to v_o and thereby estimate the rate of photorespiration at different CO₂ concentrations (Sharkey 1988). In order to calculate the rate of photorespiratory CO₂ release, it has to be taken into account that one CO₂ is released for every two oxygenation reactions (figure 1). Sharkey (1988) estimated that under ambient conditions, the rate of photorespiratory CO₂ release is about 25% of the rate of net CO₂ assimilation (A). With increasing temperatures, the specificity of rubisco for CO₂ decreases (Brooks & Farquhar 1985) and the solubility of CO₂ decreases relative to that of O₂, resulting in enhanced rates of photorespiration at high temperatures. Due to the high rates of photorespiratory CO₂ release, photorespiration is a wasteful process imposing a strong carbon drain on plants. When the rate of photorespiration becomes too high, for example, when the O₂ concentration is increased above the O₂ compensation point for a given CO₂ concentration (Tolbert et al. 1995), photorespiration leads to a depletion of carbohydrates and to accelerated senescence. On the other hand, long-term growth in low O₂ (2 kPa) to suppress photorespiration appears to be detrimental to plants and results in decreased rates of photosynthetic CO₂ assimilation (measured in air), poor plant growth and alterations in the chloroplast structure (Migge et al. 1999). It has been suggested that photorespiration is important for energy dissipation to prevent photoinhibition (Osmond 1981; Osmond & Grace 1993; Osmond et al. 1997; Kozaki & Takeba 1996; Wu et al. 1991). In addition, photorespiration can generate metabolites, such as serine and glycine, which can be exported out of the leaf (Madore & Grodzinski 1984) or used in other metabolic pathways, for example, provision of glycine for the synthesis of glutathione (Noctor et al. 1997, 1998, 1999). Since glutathione is a component of the antioxidative system in plants (Noctor & Foyer 1998), photorespiration may provide additional protection against oxidative damage in high light by supplying glycine. Thus, photorespiration, in addition to being wasteful, may also be a useful process in plants.

We have used a range of barley mutants with reduced photorespiratory enzyme activities to study the following aspects of photosynthetic metabolism: (i) the control exerted by photorespiratory enzymes on photosynthetic flux; (ii) the effect of photorespiratory metabolites on photosynthetic metabolism; (iii) regulation of the expression of photorespiratory enzymes; (iv) the occurrence of alternative photorespiratory pathways; and (v) the significance of photorespiration under stress conditions.

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2. PHOTORESPIRATORY METABOLISM

In the oxygenase reaction catalysed by rubisco, one molecule each of glycerate-3-phosphate and glycollate-2-phosphate are formed (figure 1). Seventy-five per cent of the carbon of glycollate-2-phosphate is recycled in the photorespiratory pathway (Leegood et al. 1995). In this pathway, glycollate-2-phosphate is first hydrolysed to glycollate by a chloroplastic phosphoglycollate phosphatase. After transport into the peroxisomes, glycollate is oxidized to glyoxylate by glycollate oxidase. Glyoxylate can be transaminated to glycine by serine:glyoxylate aminotransferase (SGAT) or by glutamate:glyoxylate aminotransferase. Half of the glycine molecules are converted to N⁵,N¹⁰-methylene tetrahydrofolate (THF) in the reaction catalysed by glycine decarboxylase (GDC) in the mitochondria. In this reaction, CO₂ and NH₃ are released. The other half of the glycine molecules can react with N⁵,N¹⁰-methylene THF in the serine hydroxymethyltransferase (SHMT) reaction to form serine. After transport from the mitochondria to the peroxisomes, serine is converted to RuBP by SGAT to hydroxy.pyruvate, which is reduced to glyceraldehyde by hydroxypyruvate reductase (HPR). Glyceraldehyde is then phosphorylated by glyceraldehyde kinase in the chloroplasts and the resulting glyceraldehyde-3-phosphate is converted to RuBP in the Calvin cycle. Due to the transamination of glyoxylate to glycine and the formation of NH₃ in the GDC reaction, photorespiratory carbon metabolism is intimately linked to nitrogen metabolism in the leaf. Since photorespiration proceeds at very high rates, it has been estimated that the production of NH₃ by photorespiration is an order of magnitude greater than the primary assimilation of nitrogen resulting from nitrate reduction (Keys et al. 1978). Therefore, the reassimilation of photorespired NH₃ by plastidic glutamine synthetase (GS-2) and ferredoxin-dependent glutamate synthase (Fd-GOGAT) is essential for maintaining the nitrogen status in plants. This process is necessarily very efficient: the rate of NH₃ emission in wild-type barley is about 0.01% of the rate of photorespiratory NH₃ release (Mattson et al. 1997), assuming a rate of photorespiratory CO₂ release of 25% of A.

(a) The use of mutants to study the control of photorespiratory metabolism

The involvement of most of the enzymes in photorespiratory metabolism has been confirmed by work with the respective mutants. A mutant screen was first devised by Somerville & Ogren (1979) to isolate photorespiratory mutants of Arabidopsis. It is based on the fact that...
photorespiratory mutants are conditional lethals. In high (> 0.2%) CO₂, photorespiration is suppressed and the growth of the photorespiratory mutants is indistinguishable from the wild-type. However, when the mutants are transferred from high CO₂ into air, they show severe symptoms of stress, such as chlorosis. After transferring the plants back into high CO₂, photorespiratory mutants recover. This method was also used to isolate photorespiratory mutants of barley (Kendall et al. 1983). Over the years, Arabidopsis, barley, tobacco and pea mutants with mutations in a large range of photorespiratory enzymes and transporters (phosphoglycollate phosphatase, catalase, SGAT, GDC, SHMT, NADH-dependent HPR, GS-2, Rd-GOGAT, dicarboxylate transport) have been isolated (for reviews, see Somerville 1986; Blackwell et al. 1988; Leegood et al. 1995).

While, in the long term, the homozygous photorespiratory mutants are not viable at ambient CO₂ concentrations, heterozygotes can be grown in air. We have used heterozygous mutants of GS-2, Rd-GOGAT, GDC and SGAT to study the control exerted by photorespiratory enzymes on photosynthetic and photorespiratory metabolism (Hausler et al. 1994a,b, 1996; Wingler et al. 1997, 1999a,b). The concept of control theory was developed by Kacser & Burns (1973). They showed that the control of flux through a metabolic pathway is shared by the enzymes of the pathway and that for each enzyme a control coefficient (the fractional change in the flux through a pathway divided by the fractional change in the amount of enzyme) can be calculated. As it is difficult to quantify the rate of photorespiration, the control exerted by photorespiratory enzymes on photorespiration cannot easily be determined. Accumulation of substrates of the respective reactions can, however, serve as a first indication for an impairment of flux through the photorespiratory pathway. In addition, restrictions on oxygenation of RuBP should also affect carboxylation, so that photosynthetic flux should be decreased in a manner similar to the photorespiratory flux. The effects of reduced photorespiratory enzyme activities can be expected to be most severe under conditions that lead to a high rate of oxygenation of RuBP. Such conditions are high light, low external CO₂ concentrations, high external O₂ concentrations, high temperatures and stress conditions that lead to stomatal closure and a decline in intercellular CO₂ concentrations (Cᵢ). We, therefore, studied the effect of a variety of conditions on the performance of photorespiratory barley mutants.

In contrast to the homozygous mutants, heterozygotes show only minor effects of the lowered enzyme activities on photosynthesis. Of the heterozygous mutants studied, the decrease in photosynthesis was most severe in plants with reduced activities of Rd-GOGAT (Hausler et al. 1994b). Even in moderate light and in ambient CO₂, these plants exhibited reduced rates of CO₂ assimilation. In plants with reduced activities of GDC (Wingler et al. 1997) and GS-2 (Hausler et al. 1994b), the effect on CO₂ assimilation was negligible in ambient CO₂ but became more severe in low CO₂. Plants with reduced activities of SGAT, on the other hand, did not show a significant reduction in CO₂ assimilation, even when photosynthesis was measured under conditions of high rates of photorespiration (high light and low CO₂) Wingler et al. 1999a).

However, the rates of CO₂ assimilation were reduced compared with the wild-type, when the stomata closed during moderate drought stress (Wingler et al. 1999b). Therefore, photorespiratory enzymes, such as SGAT and GDC, that are 'in excess' under normal growth conditions, can, in the long term, exert appreciable control under stress conditions that lead to increased rates of photorespiration.

In plants with reduced photorespiratory enzyme activities, the following alterations could lead to reduced rates of photosynthesis: (i) an impairment of the recycling of the carbon in the photorespiratory pathway could result in a depletion of Calvin cycle metabolites; (ii) an impairment of photorespiratory nitrogen reassimilation could result in a decline in the nitrogen status of the leaf and a reduction in the amount of photosynthetic proteins; and (iii) accumulation of photorespiratory metabolites could have a feedback effect on Calvin cycle activity.

(i) First indications that reduced rates of photosynthesis in homozygous photorespiratory mutants are due to a depletion of metabolites were obtained by providing carbon and nitrogen in metabolites that cannot be formed at sufficient rates in the mutants. For example, the supply of glutamine to a GS-2 mutant of barley (Blackwell et al. 1987), of serine to a GDC mutant of barley (Blackwell et al. 1990) or of sucrose to cell cultures of an SGAT mutant of Nicotiana sylvestris (McHale et al. 1989) partially restored photosynthetic activity. However, the pools of RuBP, which should directly affect photosynthesis, appear to be very stable in the mutants. Transfer of the homozygous barley mutant lacking GS-2 from high CO₂ into air did not lead to a decline in RuBP content (Leegood et al. 1995), and heterozygous mutants with reduced activities of GS-2, SGAT or GDC did not contain less RuBP than wild-type plants (Wingler et al. 1999b). On the other hand, other metabolites of the Calvin cycle, such as fructose-1,6-bisphosphate did decrease in the mutants, indicating a feedback regulation of rubisco activity.

(ii) Since the photorespiratory pathway and nitrogen assimilation are closely linked, one might expect that reduced photorespiratory enzyme activities could lead to a depletion of metabolically available nitrogen. Clear alterations in nitrogen metabolism became apparent in the heterozygous photorespiratory mutants: in the Rd-GOGAT mutants, the content of glutamine increased while the content of glutamate decreased (Hausler et al. 1994a), in the GS-2 mutants NH₄⁺ production increased while the content of glutamine decreased (Hausler et al. 1994a; Mattsson et al. 1997), in the SGAT mutants the content of glutamine increased while the content of serine decreased (Wingler et al. 1999a,b) (figure 2c), and in the GDC mutants the content of glycine increased under conditions of high photorespiratory flux (Wingler et al. 1997, 1999b). In plants with reduced activities of GS-2 and, more severely, in plants with reduced activities of Rd-GOGAT, the protein content in the leaves and the total activity of rubisco were reduced. It is, therefore, likely that the reduced rates of photosynthesis in plants with reduced Rd-GOGAT activities were partly caused by
lower amounts of photosynthetic enzymes due to a reduced availability of nitrogen. Plants with reduced activities of SGAT or GDC, did not, however, exhibit changes in the total protein content. In order to increase the possibility of a depletion of physiologically available nitrogen, plants with reduced SGAT activities were grown with a low supply of nitrogen. Under these conditions, the protein content was reduced to the same extent as in wild-type plants and there was no effect of reduced SGAT activities on photosynthesis (figure 2a,b), even though serine also accumulated under these conditions (figure 2c).

(iii) An accumulation of metabolites could also directly lead to a feedback regulation of photosynthetic activity. This topic has been extensively discussed by Leegood et al. (1995, 1996). There is little evidence that NH₃ accumulating in the GS-2 mutants directly inhibits photosynthesis by uncoupling of photosynthetic electron transport. This was shown by feeding glutamate to homozygous GS-2 mutants (Blackwell et al. 1987). Supply of external glutamate increased the accumulation of NH₃, while, at the same time, it restored photosynthesis to wild-type rates. Accumulation of serine is also unlikely to inhibit photosynthesis. In cell cultures of the Nicotiana sylvestris mutant lacking SGAT, an almost ninefold increase in serine had no effect on photosynthesis when sucrose was supplied as a carbon source to prevent the depletion of carbon stores (McHale et al. 1989). Of the metabolites we measured in the mutants, glyoxylate is the most likely to exert a feedback effect on photosynthesis. In in vitro studies, it had been shown that glyoxylate can inhibit the activation of rubisco (Campbell & Ogren 1990). Using heterozygous GS-2 mutants, Häsler et al. (1996) have shown that there is a negative relationship between the glyoxylate content in the leaves and the activation state of rubisco, indicating that glyoxylate can act as a feedback inhibitor of photosynthesis in vivo. Other photosynthetic metabolites that have been shown to inhibit enzymes of the Calvin cycle are glyceralde, which inhibits fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase (Schimkat et al. 1990), and glycose-2-phosphate, which inhibits triose-phosphate isomerase (Anderson 1971). In addition to inhibiting activities of certain enzymes, it has been proposed that photosynthetic metabolites might also act as signals in the regulation of the expression of photosynthetic and other enzymes.

3. REGULATION OF THE EXPRESSION OF PHOTORESPIRATORY ENZYMES

Expression of most of the photosynthetic enzymes, i.e. glycollate oxidase, catalase, HPR, SGAT, P-, H- and
T-proteins of the GDC complex and SHMT, is induced by light (Raman & Oliver 1997). Expression of the subunits of the GDC complex has been studied in detail. GDC is a mitochondrial multi-enzyme complex catalysing the conversion of glycine, NAD and THF into CO$_2$, NH$_3$, NADH and N$^3$N$^3$-methylene THF (Douce & Neuburger 1999). The GDC complex is formed from P-, H-, T- and L-proteins with an approximate stoichiometry of 2 P-protein dimers:27 H-protein monomers:9 T-protein monomers:1 L-protein dimer. In wheat leaves, the amounts of P-, H- and T-proteins were highest in the leaf tip and declined towards the base (Rogers et al. 1991), showing that their expression is under developmental control. Only the L-protein, which is also a component of the pyruvate dehydrogenase complex, was already present in the leaf base. In pea, the amounts of P-, H- and T-proteins increased when etiolated seedlings were transferred to the light (Turner et al. 1993). During the development of pea leaves, the expression of the genes encoding the P-, H- and T-proteins of the GDC complex was coordinated with the expression of the rubisco $\text{rbcS}$ genes (Vauclare et al. 1996). However, the mRNAs encoding the GDC proteins peaked before the GDC complex was formed while, at this stage, rubisco was already active. This implies that post-transcriptional regulation controls the formation of the GDC complex, possibly with a photorespiratory metabolite, such as glycine, acting as a signal. In mutants with reduced activities of GDC, we have shown that the amount of P-protein was reduced in plants that had a content of H-protein that was lower than 60% of wild-type contents, while the amounts of T- and L-proteins were normal (Blackwell et al. 1990; Wingler et al. 1997). This indicates that the mutation in this GDC mutant is probably in a gene encoding H-protein and that the synthesis of P-protein is also regulated downwards, when the formation of functional GDC complexes is limited by the availability of H-protein. After very long exposure, H-protein was also visible in the leaves of the GDC mutant on Western blots (figure 3). Dilution of extracts from wild-type leaves revealed that the amount in the mutant was about 1% of that in the wild-type. There was no difference in the content of H-protein in the roots of the GDC mutant compared with the wild-type. These results suggest that, in addition to the photorespiratory gene for H-protein, barley contains a second gene which is constitutively expressed in roots and leaves, but its function is unknown.

The other enzyme whose expression has been intensively analysed is NADH-dependent HPR. Induction of the expression of the HPR gene in cucumber by light involves a phytochrome-dependent component (Bertoni & Becker 1993). In the dark, expression of the HPR gene can be induced by cytokinin (Chen & Leisner 1985; Andersen et al. 1996). This induction has been shown to be, at least in part, due to transcriptional regulation. It has also been suggested that photorespiratory metabolites have an effect on the expression of the HPR gene. When photorespiration in cucumber plants was suppressed in high CO$_2$, the HPR mRNA decreased (Bertoni & Becker 1996). There was, however, no effect of high CO$_2$ on HPR activity in pea (Thibaud et al. 1995).

In tobacco, we found that cytokinin can prevent the senescence-dependent decline in HPR protein (Wingler et al. 1998). This was observed in naturally senescing plants, which endogenously produced cytokinin by over-expression of isopentenyl transferase, as well as in leaf discs floating on cytokinin-containing solutions. Glucose, on the other hand, accelerated the decline in HPR protein (figure 4a), and it also overrode the effect of cytokinin. Thus, the reduced expression of the HPR gene observed in high CO$_2$ (Bertoni & Becker 1996) could be part of the response of plants to sugar accumulation described as sugar-mediated gene expression (Koch 1996). In contrast, the increase in sugar contents observed in drought-stressed barley (Wingler et al. 1999b) did not lead to a decline in HPR protein. Instead, the amount of HPR protein increased in drought-stressed leaves (figure 4b). This was also the case in the SGAT and the GDC mutants, suggesting that either a general drought-related signal or a metabolite formed in the photorespiratory pathway before the GDC reaction (e.g. glycollate) could have acted as the signal.

In contrast to their cytosolic counterparts, the expression of the plastidic isoforms of GS and GOGAT (GS-2 and Rd-GOGAT) is not strongly regulated by nitrogen supply but is highly responsive to light (Hecht et al. 1988; Migge et al. 1996; Migge & Becker 1996). An involvement of photorespiratory signals in the regulation of the expression of GS-2 has been suggested by Edwards &

![Figure 4. Western blots for NADH-dependent HPR.](image-url)

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<th>gdc</th>
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<tr>
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*Phil. Trans. R. Soc. Lond. B (2000)*
Coruzzi (1989), because suppression of photorespiration in 2% CO$_2$ led to a decrease in the GS-2 mRNA in pea. Cock et al. (1991), however, were not able to detect any short-term effect on the GS-2 mRNA, when *Phaseolus vulgaris* plants grown in high CO$_2$ were transferred into air, as would have been expected if the expression was under photorespiratory control. Only in the long term, did plants growing in high CO$_2$ show a lower expression of GS-2 than air-grown plants. Growth of *Arabidopsis* or tobacco plants at a CO$_2$ concentration of 0.3%, which is probably high enough to suppress photorespiration, did not affect the amount of GS-2 mRNA compared with plants grown in air (Beckmann et al. 1997, Migge et al. 1997). Equally, there was no effect on photorespiratory metabolites in the expression of GS-2 or Rd-GOGAT is therefore questionable.

### 4. PHOTORESPIRATION IN C$_4$ PLANTS

In C$_4$ plants, CO$_2$ is first fixed as bicarbonate by phosphoenolpyruvate carboxylase (PEPC) in the mesophyll cells, resulting in the formation of the C$_4$ acids malate or aspartate. After transport of these acids to the bundle sheath cells, where rubisco is located, CO$_2$ is released in reactions catalysed by NADP-malic enzyme, NAD-malic enzyme or phosphoenolpyruvate carboxylase. Because of the high resistance of the walls of bundle sheath cells to the diffusion of CO$_2$, the CO$_2$-pumping mechanism of C$_4$ plants results in a high CO$_2$ concentration at the site of rubisco and, consequently, in a strongly reduced rate of RuBP oxygenation. There are, however, indications that photorespiration also occurs in C$_4$ plants. It has been shown that high concentrations of O$_2$ can inhibit photosynthesis, and this inhibition becomes more severe when the external CO$_2$ concentration is reduced below ambient. The optimum partial pressure of O$_2$ for photosynthesis in C$_4$ plants is, however, higher (5–10 kPa) (Dai et al. 1993, 1995, 1996; Maroco et al. 1997) than in C$_3$ plants (about 2 kPa) due to a greater demand for ATP. The oxygen sensitivity of C$_4$ photosynthesis is dependent on the plant species and on the leaf age. Dai et al. (1995) showed that photorespiration, as indicated by the inhibition of photosynthesis at supranormal O$_2$ concentrations, is higher in young and senescing than in mature leaves of maize. Within the NADP-malic enzyme and the NAD-malic enzyme subtypes of C$_4$ plants, the dicotyledon species tend to have higher rates of photosynthesis than the monocotyledon species (Maroco et al. 1997). This is probably due to a higher degree of bundle sheath leakiness for CO$_2$ in the dicotyledons. For the NAD-malic enzyme type *Amaranthus edulis*, the rate of photorespiration was estimated by measuring NH$_3$ production in the presence of the GS inhibitor phosphinothricin in air compared with 0.7% CO$_2$ (Lacuesta et al. 1997). It was calculated that the rate of photorespiration accounted for 6% of net photosynthesis.

By isolating photorespiratory mutants of *A. edulis*, Dever et al. (1995) obtained further evidence that photorespiration occurs to a significant extent in this C$_4$ species. In a screen similar to that devised by Somerville & Ogren (1979) for the isolation of photorespiratory mutant of *Arabidopsis*, it was possible to isolate *A. edulis* mutants of the C$_4$ photosynthetic pathway, as well as mutants lacking the ability to metabolize photorespiratory glycine. Similar to photorespiratory mutants of C$_3$ plants, these glycine accumulators are only viable in high CO$_2$. In air, they did not only accumulate high concentrations of glycine, but also its precursor in photorespiratory metabolism, glyoxylate (Wingler et al. 1999c). Furthermore, the production of photorespiratory NH$_3$, which is formed in the GDC reaction, was strongly reduced in these mutants (Lacuesta et al. 1997). On the other hand, the rate of photorespiration calculated from the rate of NH$_3$ production was increased to 48% of net photosynthesis in an *A. edulis* mutant lacking the C$_4$ enzyme PEPC (Lacuesta et al. 1997), probably because CO$_2$ was directly assimilated by rubisco. An increased sensitivity to O$_2$ (Maroco et al. 1998a) and a lower efficiency of electron transport for CO$_2$ assimilation (Maroco et al. 1998b) also indicated an increased rate of photorespiration in the PEPC mutant. The results obtained for the *A. edulis* mutants clearly demonstrate that, even though C$_4$ cycle activity leads to a reduction in the rate of photorespiration, this process is not completely abolished in C$_4$ plants.

### 5. THE FLEXIBILITY OF PHOTORESPIRATORY METABOLISM

The photorespiratory carbon and nitrogen cycles are not completely closed (Givan et al. 1988) and photorespiratory metabolites can be provided as substrates for other processes (Keys 1999). Glycine produced in the photorespiratory pathway can, for example, be used for the synthesis of glutathione (Noctor et al. 1997, 1998, 1999) or be exported out of the leaves (Madore & Grodzinski 1984). Some flexibility in photorespiratory nitrogen metabolism is achieved by the use of alternative amino donors. In addition to glutamate and serine, alanine and asparagine provide amino nitrogen for the synthesis of glycine (Betsche 1983; Ta & Joy 1986). This is due to the lack of specificity of the respective aminotransferases. Glutamate:glyoxylate aminotransferase also uses alanine (Nakamura & Tolbert 1983), and SGAT also catalyses asparagine:glyoxylate and serine:pyruvate aminotransferase reactions (Murray et al. 1987).

There are several possible pathways for the metabolism of glyoxylate (Igamberdiev 1989). For example, glyoxylate can be reduced back to glycollate by a NADPH-dependent HPR or an NADPH-dependent glyoxylate reductase (Tolbert et al. 1970; Kleczkowski et al. 1986, 1990) or oxidized to oxalate (Richardson & Tolbert 1961; Halliwell & Butt 1974). Glyoxylate can also be oxidatively decarboxylated to formate (Zelitch 1972; Igamberdiev et al. 1999). This decarboxylation of glyoxylate is probably a non-enzymatic reaction involving the H$_2$O$_2$ formed in the glycollate oxidase reaction (Halliwell & Butt 1974; Grodzinski 1978; Oliver 1979; Walton & Butt 1981). Since H$_2$O$_2$ is usually rapidly degraded by catalase, the rate of CO$_2$ formation by glyoxylate decarboxylation is likely to be low compared with the rate of CO$_2$ formation by GDC (Oliver 1981; Walton 1982; Yokota et al. 1985a), but assumes importance in mutants lacking SGAT (Murray et al. 1987), SHMT (Somerville & Ogren 1981) or in plants with decreased catalase activities (Brisson et al. 1998).
Formate can be activated by reacting with THF in the CI-THF synthase pathway, which provides one-carbon units for the synthesis of purines, thymidylate, methionine and formylmethionyl-tRNA (Cossins & Chen 1997). The enzymes involved in the CI-THF synthase pathway in plants are a monofunctional N\textsuperscript{5},N\textsuperscript{10}-formyl-THF synthetase (Nour & Rabinowitz 1991, 1992) and a bifunctional N\textsuperscript{3},N\textsuperscript{10}-methylene-THF dehydrogenase:N\textsuperscript{5},N\textsuperscript{10}-methylene-THF cyclohydrolase (Kirk et al. 1995). The reactions catalysed by these enzymes result in the formation of N\textsuperscript{3},N\textsuperscript{10}-methylene-THF from formate. Since N\textsuperscript{3},N\textsuperscript{10}-methylene-THF is incorporated into serine in the SHMT reaction, formate, instead of the N\textsuperscript{5},N\textsuperscript{10}-methylene-THF produced in the GDC reaction, can be used as an alternative substrate for the formation of serine (Gifford & Cossins 1982a,b; Prabhu et al. 1996). Together with the CI-THF synthase/SHMT pathway, the oxidative decarboxylation of glyoxylate to formate could, therefore, form a GDC-independent bypass to the normal photosynthetic pathway, as shown for Euglena gracilis (Yokota et al. 1985b).

By using homozygous GDC mutants of barley and A. edulis, we studied the significance of such a bypass in higher plants (Wingler et al. 1999c). In contrast to wild-type plants, the mutants showed a light-dependent accumulation of glyoxylate and formate, which was suppressed in high (0.7%) CO\textsubscript{2} after growth in air; the activity and amount of N\textsuperscript{5},N\textsuperscript{10}-formyl-THF synthetase were increased in the mutants compared with the wild-types. A similar increase in N\textsuperscript{5},N\textsuperscript{10}-formyl-THF synthetase was induced when leaves were incubated with glycine under illumination, but not in the dark. In addition, the barley mutant was capable of incorporating [\textsuperscript{14}C]formate and [2,4\textsuperscript{14}C]glycolate into serine. Since the GDC activity in the mutant (1% of wild-type activity; Wingler et al. 1997) (figure 3) was too low to support the rate of serine formation from glycolate, the formation of serine must have occurred via a GDC-independent pathway. Together, these results indicate that the mutants are able to bypass the normal photosynthetic pathway by oxidative decarboxylation of glyoxylate and formation of serine from formate, thereby partially compensating for the lack of GDC activity. The advantage of this alternative photosynthetic pathway is the absence of NH\textsubscript{3} release. In wild-type plants, the release of photosynthetic NH\textsubscript{3} is, however, very low (< 0.5 nmol m\textsuperscript{-2} s\textsuperscript{-1}) (Mattsson et al. 1997), because NH\textsubscript{3} is efficiently reassimilated by GS-2. In barley mutants with reduced activities of GS-2, on the other hand, the alternative pathway could be the mechanism by which the loss of nitrogen as NH\textsubscript{3} would be reduced (Hausler et al. 1996).

### 6. ENERGY REQUIREMENT OF PHOTORESPERATORY METABOLISM

Oxidation of 1 mol RuBP results in the formation of 1 mol glyceraldehyde-3-phosphate and 1 mol glycocolate-2-phosphate, which is converted into 0.5 mol glyceraldehyde-3-phosphate and 0.5 mol ATP and 1 mol reduced Fd (Fd\textsubscript{red}, equivalent to 0.5 mol NADPH) for the reassimilation of NH\textsubscript{3} in the GS-GOGAT cycle (table 1). Furthermore, the formation of RuBP from the 1.5 mol glyceraldehyde-3-phosphate formed per oxidation requires 2.25 mol ATP and 1.5 mol NADPH. In total, 3.25 mol ATP and 2 mol NADPH are consumed per oxygenation. Since 0.5 mol CO\textsubscript{2} is released per mol of O\textsubscript{2} fixed and assuming a photosynthetic CO\textsubscript{2} release of 25% of A (Sharkey 1988), the ratio of v\textsubscript{o} to v\textsubscript{c} rises and the energy requirement for CO\textsubscript{2} assimilation becomes even higher. In addition, the CO\textsubscript{2} released during photorespiration can be partially reassimilated, which would result in an additional energy sink.

For the alternative photosynthetic pathway involving oxidative decarboxylation of glyoxylate and formation of serine from formate, the carbon balance is the same as in the normal pathway. In this pathway, 0.5 mol ATP are required in the 10-formyl-THF synthetase reaction, and 0.5 mol NADPH in the 5,10-methylene-THF dehydrogenase reaction. This requirement is equivalent to the consumption of 0.5 mol ATP and 1 mol reduced Fd\textsubscript{red} for the reassimilation of NH\textsubscript{3} in the normal photosynthetic pathway. In contrast to the normal pathway, the alternative pathway does not include the GDC reaction, which can provide the NADH for the reduction of hydroxy-pyrurate (Hanning & Heldt 1993), so that additional reducing equivalents are required in the HPR reaction.

### 7. PROTECTION AGAINST STRESS

Because of the high energy requirement of photosynthetic metabolism, it has been suggested that photorespiration is important for maintaining electron flow to prevent photoinhibition under stress conditions (Osmond...
Takeba 1996; Osmond et al. 1981; Wu 1995. A. Wingler and others 2002. In the main, two different approaches have been employed to study the contribution of photorespiration to the protection of the photosynthetic apparatus. (i) Photorespiration has been reduced at the site of rubisco by reducing the O2 concentration to between 1% and 2%. At these O2 concentrations, the Mehler reaction is still active, while, due to its higher Km, the oxygenase reaction of rubisco is strongly reduced (Osmond 1981). (ii) In other studies, the role of photorespiration has been analysed in plants in which photorespiratory enzyme activities were reduced by feeding inhibitors, by mutagenesis or by antisense transformation. Since photorespiration per se (i.e. oxygenation of RuBP and formation of glycollate-2-phosphate) is not directly affected by reducing the activities of enzymes of the photorespiratory pathway, it is not possible to conclude from such experiments whether or not the photosynthetic apparatus of plants is protected by photorespiration. Instead, an impairment of the flux through the photorespiratory pathway can lead to photoinhibition by inhibiting photosynthetic CO2 assimilation caused by an insufficient regeneration of RuBP or by an accumulation of toxic metabolites. The best approach to study the role of photorespiration in photoprotection would be to alter the properties of rubisco (e.g. Bainbridge et al. 1995; Whitney et al. 1999) with the aim of abolishing the oxygenase activity without influencing the carboxylase activity. However, this goal has proved difficult to achieve.

In addition to the energy consumption by photorespiration, energy can also be dissipated by electron transport to O2 in the Mehler-peroxidase pathway or as heat by non-photochemical quenching. Non-photochemical quenching is linked to the formation of zeaxanthin in the xanthophyll cycle and an increased proton gradient across the thylakoid membrane (Ruban & Horton 1995). Since the Mehler-peroxidase pathway results in an increased acidification of the thylakoid lumen, it can also lead to increased energy dissipation by non-photochemical quenching (Schreiber & Neubauer 1990).

(a) Drought stress

In drought-stressed leaves, CO2 assimilation is decreased, resulting in a reduced electron consumption by photosynthesis. Consequently, the importance of mechanisms protecting the photosynthetic apparatus is increased. Under conditions of mild to moderate drought stress, the decline in photosynthesis mainly results from lower C3 caused by stomatal closure (Kaiser 1987; Cornic & Briantais 1991; Quick et al. 1992; Lal et al. 1996; Sánchez-Rodriguez et al. 1999). When the stomatal control of photosynthesis is overcome, for example, by removing the lower epidermis or by increasing the external CO2 concentration, CO2 assimilation can be restored, suggesting that, at least during mild drought, photosynthesis is not inhibited due to damage to the photosynthetic apparatus (Cornic 2000). Under these conditions, extractable activities or activation states of photosynthetic enzymes, such as rubisco, chloroplast fructose-1,6-bisphosphatase or NADP-dependent malate dehydrogenase do not typically decrease (Sharkey & Seemann 1989; Lal et al. 1996; Sánchez-Rodriguez et al. 1999; Wingler et al. 1999). In the long term, drought stress has, however, been shown to result in lower fructose-1,6-bisphosphatase activities in Casuarina equisetifolia (Sánchez-Rodriguez et al. 1999), and severe drought stress led to a decline in the amounts of sedoheptulose-1,7-bisphosphatase and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase proteins in barley (Wingler et al. 1999b). The amounts of photorespiratory enzyme proteins (proteins of the GDC complex, GS-2, SGAT) were not affected by drought stress, and the amount of NADH-dependent HPR even increased (figure 4b). In combination, the decline in C3 and sustained activities of rubisco and photorespiratory enzymes are likely to result in increased rates of photorespiration—not only relative to photosynthesis but also in absolute terms. Therefore, photorespiration could serve as an important means to maintain electron flow.

Studies conducted with different species under a variety of conditions provide partly contradictory data on the role of photorespiration during drought stress. For example, Biehler & Fock (1996) showed that gross uptake of O2 increased with increasing water deficit in wheat. The absolute rate of photorespiration, determined as the rate of glycollate formation, was, however, decreased at a water potential (ψ) of −2.6 MPa. This suggests that at this ψ, electron flow to O2 was increased due to the Mehler reaction and not due to photorespiration. Since the drought stress in this study was quite severe, reactions of the Calvin cycle might have been inhibited, which could result in reduced contents of RuBP (Sharkey & Seemann 1989) and, consequently, in lower rates of RuBP oxygenation. Brestic et al. (1995) found that electron transport in the leaves of drought-stressed Phaseolus vulgaris plants was reduced by switching from 21% to 2% oxygen, demonstrating that photorespiration serves as an electron sink in dehydrated leaves. Incubation in 2% oxygen in the light, however, did not affect the parameter Fv/Fm measured after 20 min dark adaptation. Accordingly, it can be concluded that photorespiration is not essential for protecting the photosynthetic apparatus against photoinhibition under drought stress.

In our studies, we used heterozygous barley mutants of chlorophyll fluorescence which contained approximately 50% of wild-type activities of the photorespiratory enzymes, GS-2, GDC and SGAT, to study the role of photorespiration during drought stress (Wingler et al. 1999b). These mutants have normal rates of photosynthesis in moderate light and in ambient CO2. In low CO2, on the other hand, photosynthesis is reduced in the GS and GDC mutants. The rationale behind our study was that if photorespiration is increased in dehydrated leaves, photosynthesis should decline to a greater extent in the mutants than in the wild-type with increasing drought stress, and the control exerted by the photorespiratory enzymes on photosynthesis should increase. In addition, it appeared possible that a further downregulation of photosynthetic CO2 assimilation due to restrictions in the photorespiratory flux could lead to photoinhibition.

In well-watered plants, reduced activities of GS-2, GDC or SGAT did not affect photosynthesis. Drought stress was induced by withholding water over a period of two weeks, and the decrease in leaf ψ was monitored.
daily. With decreasing $\psi$, rates of CO$_2$ assimilation declined almost linearly in the wild-type (figure 5a). In the mutants with reduced activities of photorespiratory enzymes, this decline was accelerated, resulting in lower rates of CO$_2$ assimilation at moderate drought stress. The control exerted by photorespiratory enzymes on photosynthesis was, therefore, increased in moderately drought-stressed leaves. However, under severe drought stress, the rates of CO$_2$ assimilation were equally low in the wild-type and in the mutants. The $C_i$-values determined by gas exchange measurements declined in all plants with increasing drought stress until a $\psi$ of about -1.5 MPa was reached (figure 5b). When desiccation became more severe, the relationship between $\psi$ and $C_i$ broke down, and the estimated $C_i$-values rose again. This phenomenon, i.e. constant or even increased $C_i$-values calculated from gas exchange data, is often encountered in severely drought-stressed leaves (Cornic et al. 1989; Lal et al. 1996; Sánchez-Rodríguez et al. 1999). During severe drought stress, $C_i$-values are often overestimated due to the patchiness of stomatal closure (Sharkey & Seemann 1989) and altered lateral diffusion of CO$_2$ (Lal et al. 1996). $v_i$, as calculated from the $C_i$ data and the CO$_2$ assimilation rates, decreased with increasing drought stress (figure 5c). As shown for the assimilation rates, $v_i$ was higher in drought-stressed leaves of the wild-type than in leaves of the mutants. The calculated values for $v_i$ rose until a $\psi$ of -1.5 MPa was reached (figure 5d). As a consequence of the questionable $C_i$-values, the relationship between $\psi$ and $v_o$ broke down when drought stress became more severe. Together with the lower rates of photosynthesis, the calculated values for $v_o$ indicate that during moderate drought stress, when the calculation of $C_i$ was probably still valid, photorespiration was increased. This was also confirmed by an increase in glycine contents in drought-stressed leaves of the GDC mutant (Wingler et al. 1999b).

The lower rates of photosynthesis in the heterozygous mutants were accompanied by decreased quantum efficiencies of photosystem (PS) II electron transport. This decreased electron consumption in photosynthesis and photorespiration in the mutants did not lead to a decline in $F_v/F_m$, which would have indicated chronic photoinhibition. Instead, energy dissipation by non-photochemical quenching increased. In the SGAT and GDC mutants, this was accompanied by a strong increase in the formation of zeaxanthin. As shown by Brestic et al. (1995) and Demmig et al. (1988), xanthophyll-cycle-dependent energy dissipation seems to be an important mechanism for protecting against the deleterious effect of light in drought-stressed leaves.

(b) Salt stress

Under conditions of high salinity, plants encounter similar problems to those during drought stress. Initially, photosynthesis is inhibited by closure of the stomata and the resulting decrease in $C_i$, while non-stomatal effects become limiting when the chloride concentration increases further (Walker et al. 1981; Downton et al. 1990;
As for drought-stressed leaves, severe salt stress can result in non-uniform closure of the stomata and patchiness of photosynthetic CO₂ assimilation (Downton et al. 1990). Several lines of evidence suggest that stomatal closure in moderately salt-stressed leaves leads to enhanced rates of photorespiration. The following parameters, all indicative of higher rates of photorespiration, have been shown to increase: the CO₂ compensation point (Fedina et al. 1993, 1994), the light to dark ratio of CO₂ production (Rajmane & Karadge 1986), the stimulation of photosynthesis by lowering the O₂ concentration (Walker et al. 1981; Fedina et al. 1994), the activity of glycollate oxidase (Rajmane & Karadge 1986; Fedina et al. 1993, 1994) and the formation of photorespiratory metabolites, such as glycine, serine and glycolate (Downton 1977; Rajmane & Karadge 1986; Di Martino et al. 1999). The maintenance of considerable rates of electron transport in CO₂-free air also indicates a significant occurrence of photorespiration in salt-stressed leaves (Di Martino et al. 1999). In addition to sustained rates of electron transport due to photorespiration, the formation of zeaxanthin during salt stress probably also mitigates against photoinhibitory damage, although this protection by zeaxanthin is not complete in high light (Sharma & Hall 1992).

(c) Light stress in alpine plants

Plants are particularly prone to photoinhibition when they are exposed to high light in combination with drought or low temperatures, conditions mainly encountered by plants growing at high altitudes. These plant species are often highly resistant to photoinhibition and employ different protective strategies. For Chenopodium bonus-henricus grown in the Alps, Heber et al. (1996) found that photorespiration is essential for maintaining electron flow in high light, whereas the Mehler-peroxidase pathway is insufficient to prevent photoactivation. Significant rates of electron flow were maintained when photosynthesis was inhibited by cutting the petioles or submerging the leaves in water. On the other hand, when photosynthesis and photorespiration were inhibited by feeding HCN or glyceraldehyde, the sensitivity of PS II to sunlight increased. At very low temperatures, photorespiratory metabolism is greatly reduced and cannot make a significant contribution to photoprotection. For the alpine plant Geum montanum, it was suggested that at low temperatures, cyclic electron transport around PS I results in a decrease in pH in the thylakoid lumen and, probably in combination with zeaxanthin formation, to increased energy dissipation at the site of PS II (Manuel et al. 1999). A strong dependence on the operation of the xanthophyll cycle in high light and at low temperatures has also been proposed for Soldanella alpina (Streb et al. 1998). Ranunculus glacialis, on the other hand, was less dependent on the formation of zeaxanthin, while feeding of the GS inhibitor phosphinothricin at low temperatures resulted in photoinhibition. This points to a significant flux through the photorespiratory pathway even at low temperatures. As photorespiration was, however, not blocked at the site of RuBP oxygenation, it cannot be established from this experiment that photorespiration itself has a protective function. Instead, accumulation of compounds such as NH₃ or glyoxylate or a depletion of Calvin cycle intermediates could have led to secondary effects by, for example, reducing CO₂ assimilation. In addition to the mechanisms discussed above, an increased importance of the antioxidative system comprising ascorbate, α-tocopherol and glutathione has been proposed to protect alpine plants against photo-oxidation (Heber et al. 1996; Streb et al. 1997, 1998). Since the glycine required for the formation of glutathione is mainly provided by photorespiration, photorespiration could play an additional role in the protection of alpine plants.

8. CONCLUSION

Photorespiratory metabolism is not only a wasteful process inevitably resulting from the kinetic properties of rubisco, but, precisely because of this inefficiency, is also involved in stress protection. In addition, photorespiratory metabolism can generate metabolites, such as glycine, serine or one-carbon units for other processes in plants. Abolishing photorespiration by engineering rubisco may, therefore, not necessarily lead to improved plant performance, especially under unfavourable growth conditions.

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