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Minireview

Integration of photosynthetic carbon and nitrogen metabolism in higher plants

Marie-Louise Champigny
11 rue du Docteur Roux, 92330 Sceaux, France

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Abstract

Concomitant assimilation of C and N in illuminated leaves requires the regulated partitioning of reductant and photosynthate to sustain the demands of amino acid and carbohydrate biosynthesis. The short-term responses of photosynthesis and photosynthate partitioning to N enrichment in wheat (*Triticum aestivum*, L.) and maize (*Zea mays* L.) leaves were studied in order to understand the regulatory strategy employed in higher plants. Transgenic tobacco plants (*Tobacco plumbaginifolia*) over-expressing NR or with poor NR expression were used to compare plants differing in their capacities for NO_3^- assimilation. Similar regulatory responses to NO_3^- were observed in leaves having C_4- and C_3-type photosynthesis. It was shown that the extra- C needed in the short-term to sustain amino acid synthesis was not provided by an increase in photosynthetic CO_2 fixation but rather by a rapid shift in the partitioning of photosynthetic C to amino acid at the expense of sucrose biosynthesis. The modulation of three enzymes was shown to be important in this C and N interaction, namely PEPCase (EC 4.1.1.31), SPS (EC 2.4.1.14) and NADH/NR (EC 1.6.6.1). The first two enzymes were shown to share the common feature of regulatory post-transcriptional NO_3^--dependent phosphorylation of their proteins on a seryl-residue. While PEPCase is activated, SPS activity is decreased. In contrast the NR phosphorylation state is unchanged and all N-dependent control of NR activity is regulated at the protein level. A number of arguments support the hypothesis that Gln, the primary product of NO_3^- assimilation, is the metabolite effector for short-term modulation of PEPCase, and SPS in response to N enrichment. Since a major effect of NO_3^- on the PEPCase-protein kinase activity in concentrated wheat leaf extracts was demonstrated, the hypothesis is put forward that protein phosphorylation is the primary event allowing the short-term adaptation of leaf C metabolism to changes in N supply.

Abbreviations: α-KG – α-ketoglutarate; Gln – glutamine; Glu – glutamate; NR – nitrate reductase; PEPCase – phosphoenolpyruvate carboxylase; SPS – sucrose phosphate synthase

Introduction

NO_3^- assimilation in illuminated leaves uses both chloroplast reductants (i.e., Ferredoxin) and recent photosynthate to provide carbon skeletons for amino acid biosynthesis (Fig. 1). As a consequence, there are several points of interaction between C and N assimilation at the metabolic and energy levels. Regulation is required to prevent potential competition between C and N assimilation and allow allocation of reducing power and appropriate organic precursors to the required biosynthetic pathways, in order to adapt supply to demand. This review comprehensively summarizes the present status of research exploring the effect of NO_3^- metabolism on adaptation of photosynthesis, carbon partitioning and modulation of the regulatory enzymes of N and C assimilation pathways whose activities are dependent on protein kinase activity (namely phospho*enol*pyruvate carboxylase, nitrate reductase, sucrose phosphate synthase). The discus-

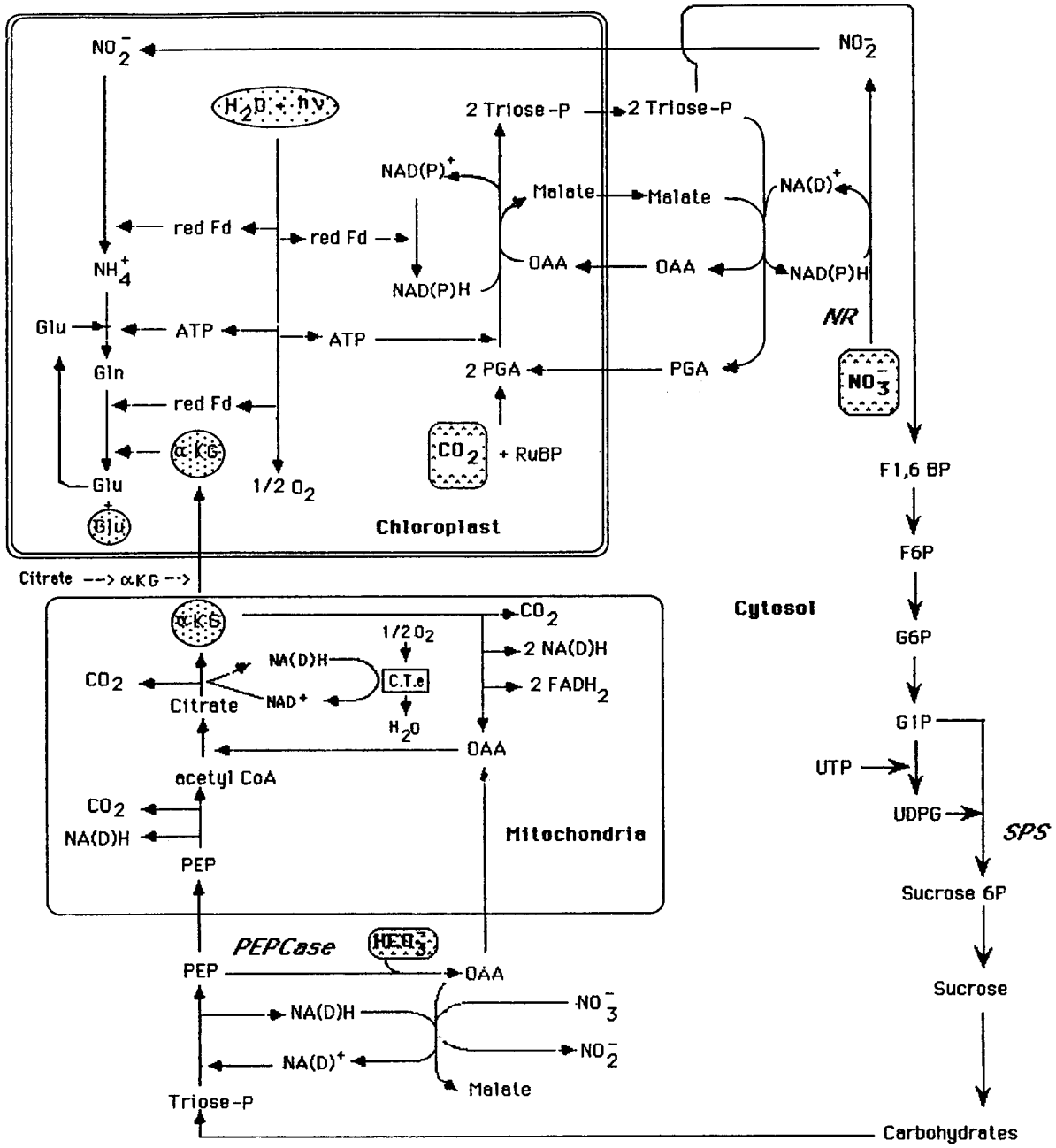

Fig. 1. A schematic representation of the major paths of C and N flow in green leaves.

sion will be limited to the short-term regulation that precedes the long-term adaptation in leaves of higher plants upon nitrogen enrichment. It will mainly draw on data from leaves of maize and wheat, and occasionally of other plants such as barley and spinach. The ability to generate transgenic tobacco plants either constitutively over-expressing NR or with poor expression of the NR gene has been very useful for comparing photosynthesis in plants differing in their capacities for NO_3^- assimilation.

N assimilation and the photosynthetic apparatus

Nitrate assimilation requires 8 electrons to reduce NO_3^- to NH_4^+ and is the highest energy consuming reaction of metabolism after CO_2 assimilation. From the average C/N ratio of plants, it can be estimated that about 20% of the photosynthetically-produced electrons are consumed for nitrate reduction. Like CO_2, NO_3^- is considered as a substrate of the Hill reaction (Warburg and Negelein 1920).

To provide energy for NO_3^- assimilation, a portion of the electrons that are used to assimilate CO_2 during photosynthesis may be instead used to reduce NO_3^- or NO_2^- (Fig. 1). Thus, when NO_3^- is assimilated by leaves, O_2 evolution in the light may be faster than when CO_2-dependent O_2 evolution alone is occuring. For example, in wild-type barley plants or NR-deficient mutants receiving ammonium as their sole N source, photosynthetic fluxes of oxygen equalled those of carbon dioxide. By contrast, wild-type plants exposed to nitrate had unequal oxygen and carbon dioxide fluxes: oxygen evolution in high light exceeded carbon dioxide consumption by 26% (Bloom et al. 1989). These results were taken to indicate that in barley, a substantial portion of photosynthetic electron transport generates reductant for nitrate assimilation rather than for carbon fixation.

The relationships between photosynthetic electron transport and the pathways of carbon and N assimilation have also been studied in C_4 plants (Khamis and Lamaze 1990; Foyer et al. 1994b). Chlorophyll *a* fluorescence analysis showed that the quantum efficiency of PS II decreased and non-radiative dissipation of excitation energy increased as CO_2 assimilation was inhibited by nitrate or nitrite. These N- compounds had no direct effect on thylakoid PS II-basal electron transport in maize (Foyer et al. 1994b). Ammonium ions weakly inhibited O_2 evolution at high concentrations. The addition of nitrogen (KNO_3, KNO_2 or NH_4Cl) caused a significant decrease in the phosphorylation state of the light-harvesting chlorophyll-*a*/*b*-binding protein of the thylakoid membranes.

N assimilation by N-depleted leaves, net photosynthesis and photosynthate partitioning

Photosynthesis provides energy and carbon for either carbohydrate or amino acid biosynthesis. Regulatory mechanisms are required to sustain the demand for NO_3^- assimilation and carbohydrate metabolism, by directing the flow of newly assimilated carbon towards sucrose and amino acid. In order to understand the strategy of these mechanisms, the responses of photosynthesis, photosynthetate partitioning and C- and N-metabolism have been studied at the onset of N assimilation when N-limited plants were supplied with abundant N nutrition. Most studies were with Maize (*Zea mays* L., cv. Contessa, INRA, France) and wheat (*Triticum aestivum* L., cv. Fidel) grown in a greenhouse on low levels of N, just sufficient to support maximal biomass production without any accumulation of NO_3^- in tissues (Khamis and Lamaze 1990). Illuminated young seedlings or detached leaves were shifted from low-N to high-N conditions by supply of 40 mM nitrate in the uptake solution. For comparative purposes, NO_2^- or NH_4^+ were occasionally supplied at equal concentrations.

Nitrogen-deficiency did not strongly alter the maximum CO_2 assimilation rate in maize at low light intensity and only slightly altered the quantum yield of CO_2 assimilation. Only at high irradiance did nitrogen limitation results in a reduction in the rate of light-saturated photosynthesis (Khamis et al. 1990). By comparison of untransformed wild-type control and transgenic *Nicotiana plumbaginifolia* over-expressing NR activity, it was observed that the decrease in N availability resulted in a decrease in net CO_2 exchange rate in both types of plants (Foyer et al. 1994c). When nitrate was supplied at high concentration to nitrogen-limited wheat or maize leaves, CO_2 assimilation was rapidly lowered but CO_2-dependent O_2 evolution was much less affected (Champigny et al. 1991) or even enhanced (De La Torre et al. 1991). The degree of inhibition of CO_2 assimilation was greatest at high irradiance and least at low irradiance (Foyer et al. 1994b).

Sucrose synthesis in illuminated detached wheat leaves was limited by short-term exposure to nitrate. A linear decrease in net sucrose synthesis was observed when the nitrate concentration in the solution increased from 0 to 100 mM NO_3^- with a correlation coefficient close to 1 (Van Quy et al. 1991b). During the first 20 min of high NO_3^- feeding to wheat leaves illuminated in the presence of $^{14}CO_2$, the decrease in (^{14}C)-sucrose synthesis was compensated for by an increase of (^{14}C) incorporation into malate and the amino acids aspartate, glutamate, serine, glycine (Champigny et al. 1991). There was enormous increase in the pools of asparagine and glutamine and the glutamine/glutamate ratio increased 300-fold.

The feeding of NO_3^-, NO_2^- or NH_4^+ to maize leaves illuminated at 250 or 700 μmol m^{-2} s^{-1} resulted in an approximate doubling of the total amino acid content within 1 h, that is at the time when maximum inhibition of CO_2 fixation was reached upon addition of NO_3^- or NO_2^- (Foyer et al. 1994b). The most marked amino acid change was observed in the Gln pool, which increased to ten times the level of the control.

When barley leaf segments or spinach leaf discs were illuminated in the presence of NH_3 in the gas phase at 30 mM, which did not inhibit photosynthesis, there was a small inhibition of sucrose synthesis and a 2–3 fold stimulation of labelling in amino acids and organic acids. In spinach leaves, there was also a decreased labelling of sucrose and starch and marked stimulation of labelling of amino acids and organic acids, but in this case, CO_2 fixation was also reduced. In both barley and spinach, the increased flux of carbon to amino acids and organic acids was accompanied by a 3–4 fold decrease in PEP and a 2-fold increase in Fru-2,6-bisP.

In sugar cane the relative capacities for NO_3^- assimilation and sucrose synthesis was shown to be a factor involved in the control of sucrose synthesis by NO_3^-, and in the rapidity with which C metabolism is adjusted to N assimilation (Pelaez Abellan et al. 1994).

It is obvious that in all leaves hitherto studied, sucrose synthesis was rapidly lowered upon the addition of N to N-deficient leaves, while the rate of C assimilation was less affected or remained the same. Thus, upon supply of inorganic N to N-deficient leaves, the extra C needed in the short-term to sustain N assimilation, was not supported by any increase of photosynthesic C fixation, but rather by a rapid shift in the partitioning of photosynthetic carbon to amino acid at the expense of carbohydrate biosynthesis.

N assimilation and post-translational modification of the key regulatory enzymes: Phospho*enol*pyruvate carboxylase, sucrose phosphate synthase, nitrate reductase

To date, three enzymes appear to be important in the C and N interaction, namely PEPCase (EC 4.1.1.31), NADH/NR (EC 1.6.6.1), and SPS (EC 2.4.1.14). They are regulatory enzymes known as key enzymes of the anapleurotic CO_2 fixation, nitrate assimilation, and sucrose biosynthesis respectively. The three enzymes occur as soluble proteins in the cytoplasm, though SPS and NR may also associate with membranes to some extent.

The increased demand for carbon skeletons created in leaves by high rates of NO_3^- assimilation was shown to be met by diversion of the fixed carbon from carbohydrate (sucrose) synthesis to the anapleurotic pathway catalyzed by PEPCase for production of amino acids and organic acids (Latzko and Kelly 1983). The rate of nitrate reduction is considered to be the limiting factor for growth, development and protein production in plants (Solomonson and Barber 1990). The role of SPS in controlling the flux of sucrose synthesis in vivo, and maintaining the required balance among photosynthetic rate, the rate of sucrose export and starch accumulation within the chloroplasts, has been documented in several species.

The PEPCase and SPS activities of wheat on low-NO_3^- diet increased 2.3-fold within 10 to 30 min after detached leaves were transferred to the light (Van Quy et al. 1991a). When illumination was concomitant with supply of 40 mM NO_3^- the light-dependent activation of PEPCase occured more rapidly than with illumination alone and activity increased up to 3.8 times that of the dark control (Fig. 2). The time-course of PEPCase activity was very similar to that of NO_3^- uptake (Van Quy et al., 1991a). In contrast, there was an immediate decrease of the leaf SPS activity in the V_{max} assay, as well as in the limiting assay, upon supply of NO_3^- (Van Quy et al. 1991a). Similar responses to high NO_3^- were observed with maize leaves (Fig. 2; Foyer et al. 1994c).

The maximum level of extractable NR activity increased in maize leaves and wild-type tobacco leaves upon supply with NO_3^-, as compared with supply with an equal concentration of KCl (Fig. 3). NO_3^- initiates a sequence of events in maize leaves, by which the steady-state levels of NADH:NR are increased. NR mRNA increases to 50% of the maximum level of NR transcript in 2 h, but increase in NR protein levels and finally of NR activity occur after at least 12 h of induction (Gowri and Campbell 1989; Hamat et al. 1989; Melzer et al. 1989). N-induced short-term changes in NR activity also involve changes in protein turnover since post-translational modification of the enzyme is unresponse to NO_3^-.

As PEPCase, SPS, and NR all respond rapidly to high NO_3^- feeding, their synchronous modulation allows short-term regulation of the relative partitioning of newly fixed carbon between sucrose and amino acid synthesis, and enables the plant to support the car-

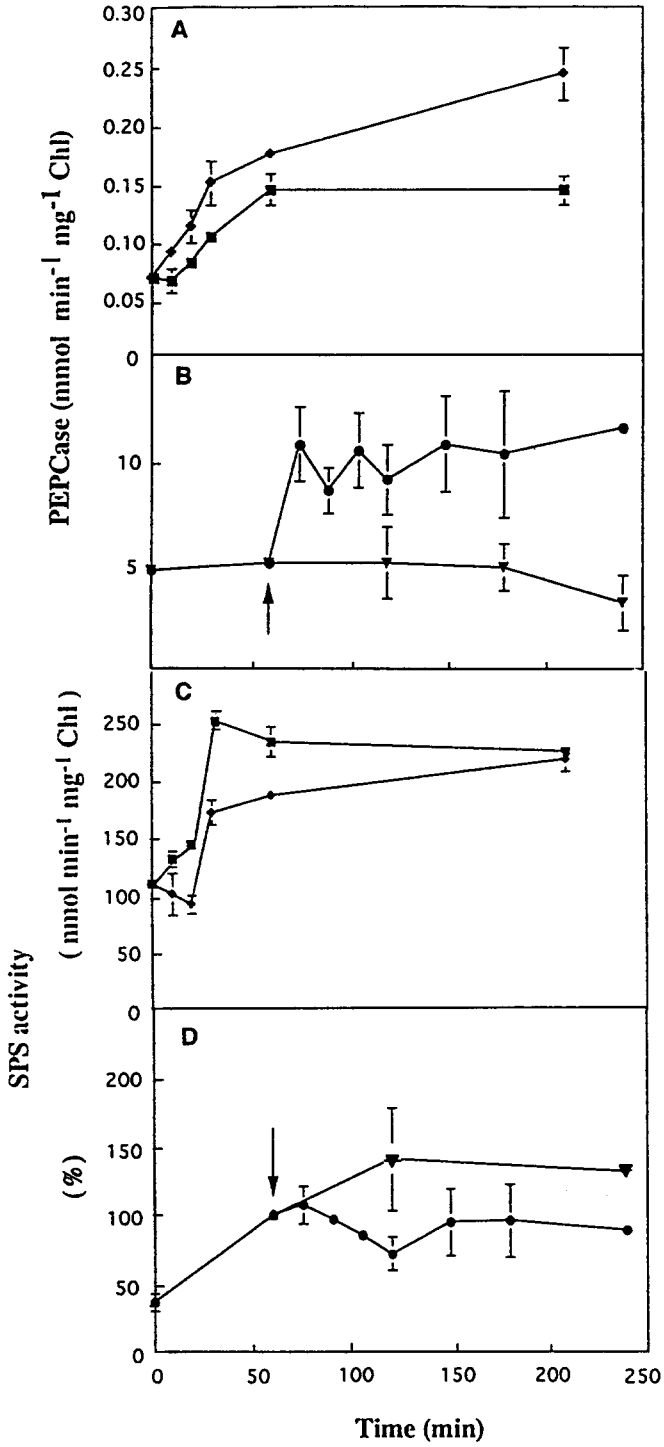

Fig. 2. The effect of 40 mM NO_3^- on maximal extractable PEPCase (A, B) and SPS (C, D) activities from (A, C)) wheat leaves and (B, D) maize leaves. In (A, C) wheat seedlings were kept in darkness for 16 h on N-free solution before the youngest mature leaves were detached and transferred to either N-free solution (■) or 40 mM NO_3^- (◆). In (B, D) maize seedlings were incubated in N-deficient media. The subsequent addition of NO_3^- (●) or Kcl (▼) is indicated by the arrow. Irradiance (A: 520 mmol m^{-2} S^{-1}; B: 700 mmol m^{-2} s^{-1}) commenced at time 0. The results are the mean ± SE (n = 3).

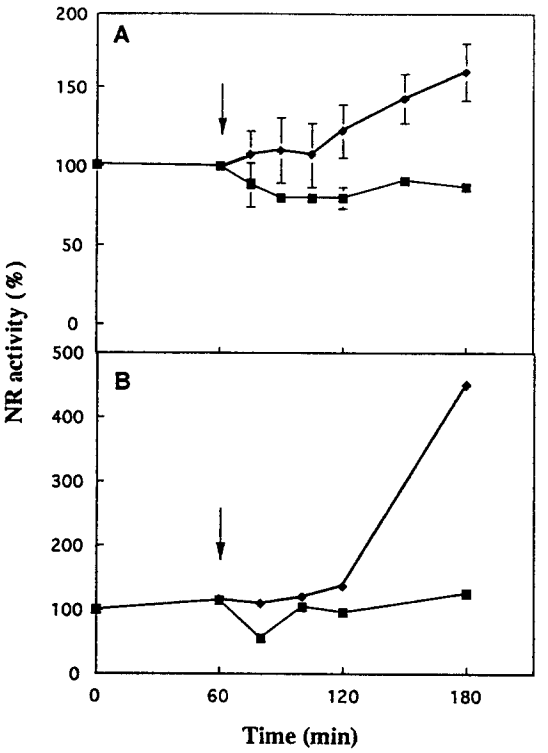

Fig. 3. The effect of addition of NO_3^- on total extractable NR (in the presence of EDTA) activity from maize (A) and N. *plumbaginifolia* (B) leaves. Excised maize leaves were incubated in nutrient solution containing 0.05 mM NO_3^- at 700 mmol m^{-2} s^{-1}irradiance. NO_3^- (♦) (40 mM maize or 30 mM tobacco) or KCl (■) (40 or 30 mM, respectively) was added to the bathing solution at the point indicated by the arrow.

bon demand associated with N assimilation (Melzer and O'Leary 1987; Champigny and Foyer 1992).

N assimilation and the phosphorylation level of the key regulatory enzymes

There is increasing evidence that a common mechanism for the modulation of the activity of PEPCase, SPS and NR may be reversible protein phosphorylation on specific seryl residues. It was well established that the light-modulation of PEPCase permitting the concentration of carbon dioxide in C_4 photosynthetic tissues and in CAM plants involves reversible phosphorylation on specific seryl residues of the protein and resulted in lower sensitivity of the enzyme to malate inhibition and higher sensitivity to glucose-6-P activation (Jiao et al. 1991a; 1991b). The phosphorylated form of the enzyme is more active than the non-phosphorylated form, with increase of V_{max} and no change of $Km_{(PEP)}$ (Jiao and Chollet 1988). In plants with the C_3 mode of photosynthesis, PEPCase is responsible for the anapleurotic replenishment of the tricarboxylic acid cycle, largely through the use of atmospheric CO_2 (Melzer and O'Leary 1987). In vivo ^{32}P labelling experiments carried out with wheat leaves showed that high-NO_3^- nutrition enhanced the light-induced phosphorylation state, together with the catalytic activity of the protein PEPCase (Van Quy et al. 1991a). This regulatory phosphorylation occurs most probably at the invariant Ser residue near the N terminus of the plant protein (Duff and Chollet 1995).

NR activity has been measured in extracts from spinach leaves (*Spinacia oleracea* L.) in response to rapid changes in illumination. Measured in buffer containing Mg^{2+}, NR from leaves decreased in the dark and increased again upon illumination. These observed short-term changes were concomitant with reversible phosphorylation (in the dark)/dephosphorylation (in the light). A crude correlation was shown between the degree of phosphorylation of some seryl residues of the NR subunit and the catalytic activity of the enzyme (Huber et al. 1992a). NR is known to be influenced in vivo by nitrate availability in a complex interaction between the regulation of gene transcription, post-translational modulation (Cheng et al. 1986; Galangau et al. 1988; Deng et al. 1991).

The activity of SPS is regulated in an analogous manner. Increasing rates of photosynthesis leads to a 'light-activation' of SPS (Stitt et al. 1988). The light-activation involves protein dephosphorylation (Huber et al. 1989). As for NR, light/dark regulation of SPS occurs as a result of dephosphorylation/phosphorylation respectively of two regulatory seryl residues (Huber and Huber 1992). Another similarity between the two substrate proteins NR and SPS is their dephosphorylation by protein phosphatase(s) of the same class (type 2A) and in the same subcellular compartment. However, it is clear that activation of NR and SPS differs significantly in terms of regulation. Several divalent anions (HPO_4^{--}, sulfate and tungstate) inhibit SPS but not NR. In general, salts inhibit the activation of SPS in vitro (Huber and Huber 1990), whereas NR activation is enhanced by high ionic strength.

Phosphorylation of specific seryl residues of PEPCase and SPS, but not NR modulates their activity according to the availability of NO_3^-. While PEPCase is activated by in vivo phosphorylation, both SPS and

NR are activated in vivo by dephosphorylation when leaves are illuminated or fed with NO_3^-. Modulation by post-translational protein phosphorylation of PEPCase and SPS in response to the addition of N was interpreted as the basic coordination-mechanism of the regulation of C and N assimilation in leaves. Since NR is also regulated by phosphorylation (i.e., decreased activity with increasing protein phosphorylation level), modulation of the NR protein kinase by NO_3^- would cause short-term inactivation of NR that would in turn limit flux through the N-assimilation pathway (Foyer et al. 1994b). This would, in effect counteract the positive modulation of carbon flow to N-assimilation caused by phosphorylation of PEPCase and SPS and hence it would not be beneficial. The absence of NO_3^- mediated regulation of the NR protein kinase is therefore a strategic advantage in the C/N interaction at this point.

It must be remenbered that NO_3^- plays an important role in the regulation of PEPCase and NR at the transcriptional level. Other short-term mechanisms of post-transcriptional regulation may precede or be superimposed upon the NO_3^--dependent transcriptional regulation.

N assimilation and regulation of the protein kinase(s). Role of Gln

Based on the responses of PEPCase and SPS in leaves to light and NO_3^-, role of NO_3^- as either an inhibitor of P-protein phosphatase(s) or activator of protein kinase(s) must now be assessed in comparison with the now well-elucidated mechanism of the regulatory light-dependent phosphorylation of PEPCase, SPS, and NR (Huber and Huber 1990; Huber et al. 1992a, 1992b; Jiao and Chollet 1991; Jiao et al. 1991a, 1991b; Spill and Kaiser 1994). In the presence of NO_3^-, the phosphorylation state of both PEPCase and SPS increased. This caused activation of the former enzyme and inhibition of the latter. It was suggested that NO_3^- modulated the relative protein kinase/protein phosphatase ratio favouring phosphorylation of both enzymes. The question of whether NO_3^- assimilation stimulated the protein kinase(s) or inhibited the phosphatase(s) was investigated with wheat. Detached N-deficient leaves were treated in vivo with mannose which inhibits the protein kinase activity, or okadaic acid specific inhibitor of the type 2A phosphatases and were then fed NO_3^- at high concentration in the light. Mannose lowered the activity of PEPCase and increased the previously deactivated SPS to the level of PEPCase and SPS in leaves on N-free solution. In contrast, treatment with okadaic acid enhanced PEPCase activation and SPS deactivation more in leaves supplied with NO_3^- than in leaves on N-free solution. The results were taken to suggest that high NO_3^- nutrition enhanced the activity of the light-modulated protein kinase(s) but did not affect the protein phosphatase(s) (Van Quy and Champigny 1992). They led to the concept that nitrate functions as a signal metabolite activating the cytosolic protein kinase(s), thereby modulating the activities of at least two of the key enzymes of assimilate partitioning, namely PEPCase and SPS, and redirecting the flow of carbon away from sucrose biosynthesis toward amino acid synthesis (Champigny and Foyer 1992). Recently, the wheat leaf PEPCase-PK activity was measured in vivo in extracts from wheat leaves that had undergone different treatments. A rather dramatic increase in PEPCase-PK activity was observed with illuminated leaves fed exogenous nitrate (Duff and Chollet 1995). These results comfort the concept that nitrate functions as a signal metabolite activating the cytosolic protein kinase(s).

A number of arguments support the hypothesis that the primary products of N assimilation Gln and Glu (or the Gln:Glu ratio) are the important metabolite effectors for short-term NO_3^--dependent modulation of PEPCase, NR, and SPS :

i Gln, rather than NO_3^-, NO_2^-, NH_3, was the deduced effector in experiments where wheat leaves were fed with inorganic N, amino acids or with specific inhibitors of inorganic N assimilation. It is only by increase of the in vivo Gln level (by feeding the leaves with Gln or with azaserine, an inhibitor of Gln amide transfer reaction) that short-term enhancement of light-dependent activation of PEPCase and limitation of net sucrose synthesis similar to the effect caused by NO_3^- were observed. The limitation of sucrose synthesis was inversely related with the concentration of Gln fed to leaves. Conversely Glu or methionine sulfoximine (inhibitor of Gln synthetase) decreased light-dependent PEPCase activation and restored net sucrose synthesis to the level of the N-free leaves in NO_3^--fed leaves.

ii The levels of both Gln and Glu were drastically changed when leaves were transferred from low to high N-nutrition. Changes in these metabolite levels were always concomitant with modulation of PEPCase (Foyer et al. 1994a; Thi Manh et al. 1993);

iii Like ATP and/or AMP in presence of Mg^{2+} and sucrose, Gln mimics the light effect on NR (MacKintosh 1992; Vincentz et al. 1993);

iv In *Nicotiana plumbaginifolia* transgenic plants, PEP carboxylase activity increased significantly in the low NR expressors containing a 1:40 Gln:Glu ratio compared with the high NR expressors containing a 1:2 Gln:Glu ratio (Foyer et al. 1994a);

v In addition to the role as effector at the post-translational level, Gln causes a strong activation of enzymes, acting at all levels of regulation. In wheat as in maize, expression of PEPCase genes was enhanced by Gln and decreased by Glu (Sugiharto and Sugiyama 1992; Sugiharto et al. 1992). In *Nicotiana tabacum*, the maximum and minimum levels of mRNA coding for NR coincided respectively with a low content and high content of Gln (Deng et al. 1991)

Since Gln exerted a positive control on PEPCase expression and a negative control on NR expression, it was suggested that Gln modulates protein kinase activity. The hypothesis was tested in vitro using purified PEPCase and partially purified protein b kinase from wheat leaves. Activation of the wheat leaf PEPCase by phosphorylation was enhanced by Gln and lowered by Glu suggesting that PEPCase-protein kinase might be the target of the glutamine and glutamate action (Thi Manh et al. 1993).

Discussion

Much emphasis is now placed on short-term modulation of enzymes of carbon and nitrogen metabolism as a crucial point of interaction to changes to nitrate supply in leaves. Similar responses were observed in leaves having the C_3-type (wheat) and C_4-type (maize) carbon metabolism. Post-translational modulation of PEPCase and SPS contributes to the synchronisation of N assimilation, C fixation, and C partitioning. Upon the transition from N-deficient to N-sufficient conditions short-term regulation in leaves precedes the long-term de novo synthesis of the enzymes.

The anapleurotic function of PEPCase and its key role in N assimilation has been known for a long time (O'Leary 1982). As shown in the present review, the mechanism of its control for rapid participation in the supply of carbon skeletons stems from the five points outlined in Fig. 4: (i) the short-term control is achieved by post-translational modulation and not by PEPCase protein turnover; (ii) the PEPCase protein is subject to phosphorylation. Enhancement of the phosphorylation level converts the low-activity form to the high-activity form and lowers its sensitivity to malate; (iii) phosphorylation of the PEPCase protein increases upon shifting leaves from limited-N to sufficient-N nutrition; (iv) the post-translational modulation by phosphorylation of the protein depends on the protein kinase activity; (v) Gln and Glu (in other words the Gln:Glu ratio) are the metabolites that regulate positively and negatively respectively the PEPCase-protein kinase activity and hence the phosphorylation status and activity of PEPCase.

Beside PEPCase, SPS contribute also to the synchronisation of N and C assimilation. That the responses to N supply of PEPCase and SPS are opposite is consistent with their distinct roles in amino acid and carbohydrate biosynthesis. The mechanism of post-translational modulation of SPS is very similar to that of PEPCase. It was shown to respond to the points (i) to (iv) cited above, with the difference that the phosphorylated SPS is the low-activity form (Fig. 4). Thus activation of the SPS-protein kinase by Gln (NO_3^- assimilation product) helps redirect the carbon flow away from sucrose synthesis toward amino acid synthesis.

Like SPS, the phosphorylated form of NR is a low-activity one. Inhibition of NR activity in leaves supplied with NO_3^- is hardly conceivable. It is now accepted that the rate of NO_3^- reduction is a major regulatory factor of N assimilation in leaves (Foyer et al. 1994c). Phosphorylation of the NR-protein is interpreted as a means of limiting the substrate-dependent activation of NR. Short-term protein turnover changes cause increases in NR activity allowing up-regulation of N assimilation.

It is important to mention that requirements for NH_4^+ and NO_3^- assimilation differ in plants and algae. In N-limited *Selenastrum minutum* for instance, nitrogen enrichment causes suppression of photosynthetic CO_2 fixation, a decreased flow of fixed CO_2 to carbohydrates, stimulation of respiration in the light as in the dark, with mobilization of starch to provide carbon to amino acid synthesis (Weger et al. 1989). Inhibition of the cytosolic gluconeogenesis is through N-dependent regulation of the thiol-regulated enzymes like fructose-1,6-bisphosphatase. In addition, redox regulation of the chloroplastic fructose-1,6-bisphosphatase and other enzymes of the reductive pentose phosphate pathway enzymes ensures against futile cycling of carbon between the chloroplastic reductive and oxidative pentose phosphate pathways.

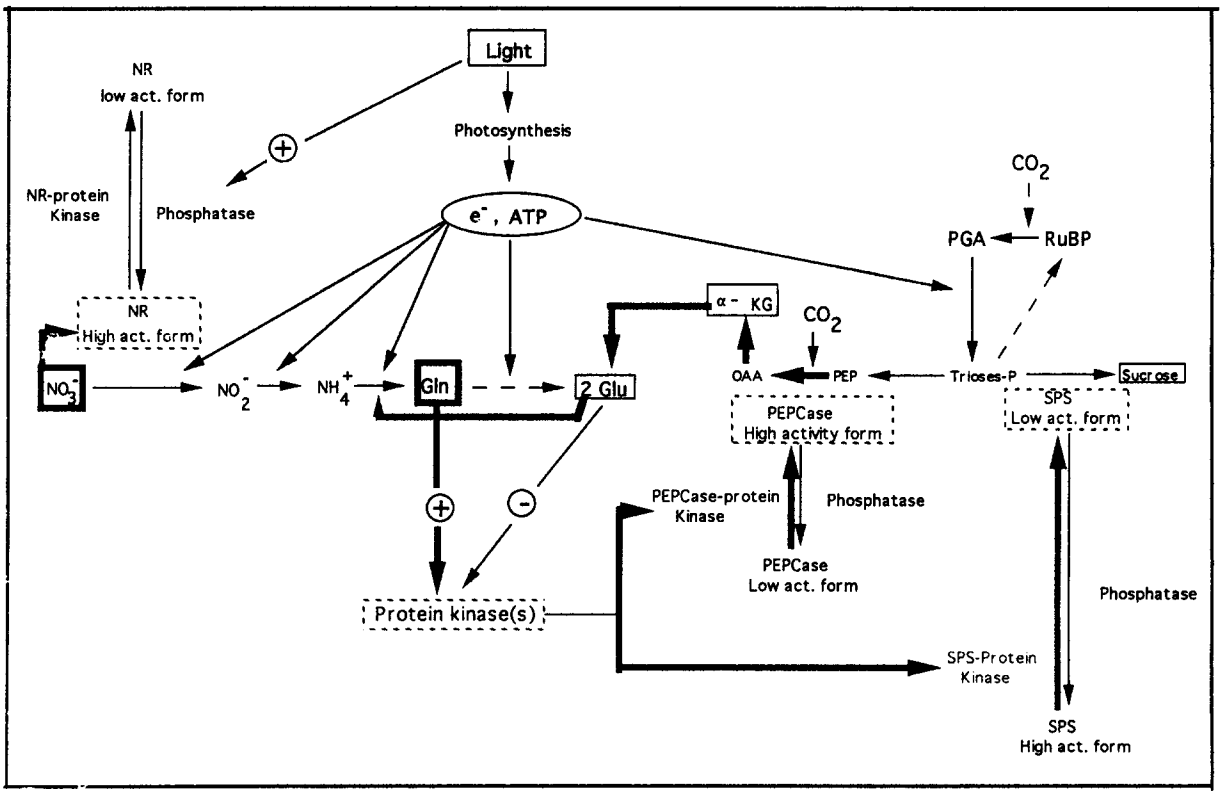

Fig. 4. A scheme for the short-term co-regulation of C and N metabolism in response to transfer of leaves from deficient to sufficient supply of NO_3^-. (⊕) positive effect of Gln; (⊖) negative effect of Glu; (⟶) activation of NR, protein kinase(s), PEPCase and deactivation of SPS owing to enhancement of the respective protein kinase(s) by increased Gln and decreased Glu during (NO_3^-) activated NO_3^- assimilation and high α-KG demand in illuminated leaves; (→) Metabolic pathways.

The hypothesis of a major role for phosphorylation/dephosphorylation of PEPCase and SPS proteins for the short-term N-dependent coordination of leaf C and N metabolism is now emerging. It is now well established that at least in wheat leaf, the N-dependent activation of the protein kinase may be a prequisite for activation of PEPCase (Duff and Chollet 1995). Separation of several distinct kinases has not yet been performed and there is no clear evidence for the participation of 3 specific kinases to the phosphorylation of PEPCase, SPS and NR protein respectively. Nevertheless, the mechanism of NR phosphorylation is different from that of PEPCase and SPS. Phosphorylation of NR was shown to be a multicomponent process which takes place in two steps (Spill and Kaiser 1994; MacKintosh et al. 1995). Two proteins are required for inactivation of NR, the protein kinase and the NR inhibitor protein: Phosphorylation catalyzed by the kinase is a prerequisite for interaction of the NR inhibitor protein.

The mechanism of the signal-transduction from protein-kinase to the in vivo C/N interaction remains to be established in view of the now well established mechanism of the light-dependent regulatory phosphorylation of C_4 leaf PEPCase (Bakrim et al. 1992; Pacquit et al. 1993). Questions to be addressed concern: (i) Determination of the precise nature of the signal (Gln or other), the concentration of Gln and Glu at the site of action, the sensitivity of protein-kinases or phosphatases to the metabolite signal. (ii) Purification and characterization (specificity, identification and location of the phosphorylation domain in the protein) of the PEPCase-, SPS-, NR-protein kinase(s) or phosphatase(s). (iii) Determination of the protein-kinase compartmentation. (iv) Identification of the specific nature of the link between the 'NO_3^-' transducing steps and the protein kinase that achieves the final physiological response.

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