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Glycine Decarboxylase: Protein Chemistry and Molecular Biology of the Major Protein in Leaf Mitochondria

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The four component proteins of the glycine decarboxylase multienzyme complex (the P-, H-, T-, and L-proteins) comprise over one-third of the soluble proteins in mitochondria isolated from the leaves of C_3 plants. Together with serine hydroxymethyltransferase, glycine decarboxylase converts glycine to serine and is the site of photorespiratory CO_2 and NH_3 release. The component proteins of the complex are encoded on nuclear genes with N-terminal presequences that target them to the mitochondria. The isolated complex readily dissociates into its component proteins and reassociates into the intact complex *in vitro*. Because of the intimate association between photosynthesis and photorespiration, the proteins of the complex are present at higher levels in leaves in the light. The expression of these genes is controlled at the transcriptional level and the kinetics of expression are closely related to those of the small subunit of Rubisco. Deletion analysis of fusions between the promoter of the H-protein of the complex and the reporter gene β-glucuronidase in transgenic tobacco has identified a region responsible for the tissue specificity and light dependence of gene expression. Gel shift experiments show that a nuclear protein in leaves binds to this region. Glycine decarboxylase has proven to be an excellent system for studying problems in plant biochemistry ranging from protein–protein interactions to control of gene expression.

KEY WORDS: Glycine decarboxylase; mitochondria; photorespiration; gene expression; light control.

INTRODUCTION

In 1955 Decker recognized that most plants exhibited a very high rate of respiration immediately after the lights were extinguished (Decker, 1955). He correctly interpreted this respiratory burst after the lights went out as a carryover of a rapid rate of respiration that was occurring in the light. This light-dependent respiration has come to be known as photorespiration. In the 1960's, Zelitch realized that photorespiration had characteristics that were very similar to the synthesis of an unusual two-carbon organic acid, glycolate (Zelitch, 1971). By the time that Ogren's group had discovered that glycolate (or more strictly its precursor—phosphoglycolate) was formed by an unknown oxygenase activity of RuBP carboxylase (Bowes *et al.*, 1971), other workers, mainly in Tolbert's laboratory, had deduced the mechanism of glycolate metabolism, the so-called C_2 cycle (Husic *et al.*, 1987; Lorimer and Andrews, 1981; Tolbert, 1979). The reactions were predicted from metabolic studies and most of the enzymes were quickly isolated and characterized; all except one enzyme that just refused to cooperate, glycine decarboxylase.

Several groups had shown that leaf mitochondria were able to convert glycine to serine (Woo and Osmond, 1976; Moore *et al.*, 1977). The reaction appeared to be 2 glycine + $NAD^+ \rightarrow$ serine + NH_3 + CO_2 + NADH. The NADH was reoxidized by mitochondrial electron transport chain to reduce $^1/_2$ O_2 to H_2O and was coupled to the synthesis of ATP. Based on work done earlier with animal mitochondria, the

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reaction was assumed to be catalyzed by two different enzymes, glycine decarboxylase (GDC)[2] and serine hydroxymethyl transferase (SHMT) (Kikuchi, 1973).

Glycine Decarboxylase Reaction

$$\text{Glycine} + NAD^+ + THF \rightarrow N^5,N^{10}\text{-methylene-THF} + CO_2 + NH_3 + NADH$$

Serine Hydroxymethyltransferase Reaction

$$\text{Glycine} + N^5,N^{10}\text{-methylene-THF} + H_2O \rightarrow \text{serine} + THF$$

Overall Reaction

$$2\ \text{Glycine} + NAD^+ + H_2O \rightarrow \text{serine} + CO_2 + NH_3 + NADH$$

While data accumulated that this was the site of photorespiratory CO_2 and NH_3 release, progress toward identifying the enzymes involved was slow. Mitochondria with very high rates of glycine oxidation could be isolated from pea and spinach leaves. But when the mitochondria were lysed, all GDC activity was lost. Several of us trying to work with this protein came to the not unreasonable, but incorrect, conclusion that the GDC was membrane bound and that like many membrane-bound proteins, it lost activity when solubilized (Moore *et al.*, 1977; Sarojini and Oliver, 1983).

The answer actually proved to be much simpler. For reasons I will discuss later, the activity of the enzyme is very sensitive to enzyme concentration. At low concentrations the complex falls apart and the enzyme activity is very low. Once we realized this, it was relatively simple to solubilize (at first by forming acetone powders but later by a freeze-thaw step) the protein and to begin its purification (Sarojini and Oliver 1983; Walker and Oliver, 1986a; Bourguignon *et al.*, 1988).

PROTEIN CHEMISTRY

Glycine decarboxylase is formed from four different component proteins that must function together to catalyze the rapid continuous decarboxylation of glycine (Oliver, 1994). The four proteins are called the P-protein (so named because it has a bound pyridoxal 5-phosphate cofactor), the H-protein (originally identified as a hydrogen carrier and later shown to contain the cofactor lipoic acid), T-protein (a tetrahydrofolate transferase), and L-protein (the flavoprotein lipoamide dehydrogenase). We have spent a great deal of time trying to stabilize the resulting complex so that it could be purified free from the other proteins of the mitochondrial matrix and have been completely unsuccessful. When the concentration of soluble proteins released from the mitochondria decreases below about 0.25 mg per ml, the complex falls apart into its component proteins (Oliver *et al.*, 1990). The exact same thing appears to happen when fractionation techniques like gel filtration, ion exchange chromatography, or ultracentrifugation are applied to the complex (Sarojini and Oliver, 1984); it falls apart. As a result, no one has ever reported the purification of the intact complex. Instead methods have been developed that purify the four separate component proteins that are then reconstituted to reform the active complex (Walker and Oliver, 1986a; Bourguignon *et al.*, 1988).

Purification of the individual component proteins required a range of assays that are dependent on one or more of these proteins. The L-protein can be measured directly. It will catalyze the transfer of electrons from dihydrolipoic acid to NAD^+, a reaction that can be followed in a spectrophotometer (Bourguignon *et al.*, 1988). The H-protein can be assayed by its ability to transfer electrons to DTNB in the presence of an excess of lipoamide dehydrogenase and NADH to keep the H-protein reduced (Oliver *et al.*, 1990). P-protein can be measured because in the presence of excess H-protein it will catalyze the exchange of $^{14}CO_2$ with the carboxyl carbon of glycine yielding [1 − ^{14}C] glycine (Sarojini and Oliver, 1983; Walker and Oliver, 1986a). Once you have isolated and purified L-protein, H-protein, and P-protein they can be used to assay for T-protein. In the presence of all four subunits, the complex will release $^{14}CO_2$ from [1 − ^{14}C] glycine (Walker and Oliver, 1986a). This reaction requires all of the cofactors for the reaction. Since the PLP tends to partially dissociate from the P-protein during purification, its addition will stimulate the reaction. NAD and THF act as the terminal acceptors for the electrons removed during glycine oxidation and the methylene carbon left after the carboxyl carbon and α-amino groups are lost, respectively. In order for the physiological reaction to occur, SHMT must be added to react the methylene-THF formed by glycine oxidation with a second glycine to form serine.

P-protein from peas is 971 amino acids-with a predicted molecular mass of 105 kDa (Turner *et al.*,

[2] Abbreviation: GDC, glycine decarboxylase complex; *gdc*H, gene for the H-protein of glycine decarboxylase; *rbc*S, small subunit of Rubisco; Rubisco, ribulose bisphosphate carboxylase/oxygenase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

1992b). It is synthesized as a preprotein with an 86 amino acid extension that directs the precursor form to the mitochondria and is removed following uptake into the matrix. Sequence comparisons with other species show a well-conserved leucine zipper that is probably responsible for homodimerization of the P-protein monomers.

H-protein is 131 amino acid (13.9 kDa) with a 34 amino acid presequence (Kim and Oliver, 1990; Macherel *et al.*, 1990; Srinivasan and Oliver, 1992). The lipoic acid is apparently attached to lys^{63}. In animal mitochondria lipoic acid is added to the mature protein after transport to the mitochondrial matrix and processing (Fujiwara *et al.*, 1990). There is one gene for H-protein in *Arabidopsis* and peas. Purified H-protein can behave as a monomer (most commonly), dimer, or tetramer on gel filtration columns depending on buffer conditions. The H-protein from peas is the only component of the complex that has been crystallized (Sieker *et al.*, 1991) and a high-resolution x-ray structure has been reported (Pares *et al.*, 1994).

T-protein is 408 amino acids (41 kDa) with a 30 amino acid presequence (Bourguignon *et al.*, 1993; Kopriva and Bauwe, 1994) and shows no sequence homology with known proteins. The protein in the complex is a monomer.

L-protein from peas is 470 amino acids (50 kDa) that is synthesized with a 31 amino acid presequence (Bourguignon *et al.*, 1992; Turner *et al.*, 1992c). The mature protein is a dimer. Flavin and pyrimidine nucleotide binding sites have been identified by homology with the equivalent protein from yeast. The L-protein of GDC is also the E3 subunit (lipoamide dehydrogenase) of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase with apparently one gene producing polypeptides for all three complexes. Interestingly, plastids also contain pyruvate dehydrogenase and the E3 subunit is either encoded by the same gene or one very closely related (Camp and Randall, 1985).

REACTION MECHANISM

At first glance, the mechanism of the GDC reaction appears to be rather complicated. But if we take it one step at time, we will see that it is composed of four rather straightforward reactions that are tied together within this multienzyme complex (Fig. 1). The reaction begins with the formation of a Schiff base between the carbonyl group of the pyridoxal 5-phosphate cofactor on the P-protein and α-amino group

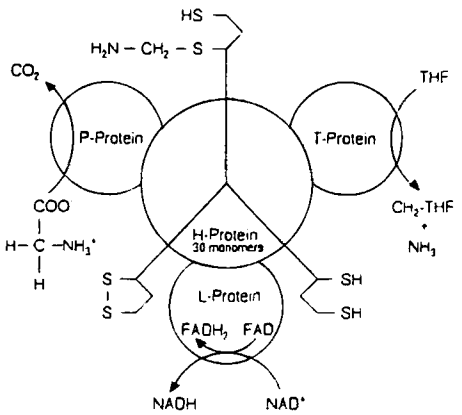

Fig. 1. Reaction mechanism for the glycine decarboxylase multienzyme complex. The subunit ratio of the different component proteins is 2 P-protein dimers:27 H-protein monomer:9 T-protein monomers:1 L-protein dimer (Oliver *et al.*, 1990).

of glycine. The withdrawal of an electron from the α-carbon and the α-carboxyl group of the glycine molecule begins the decarboxylation reaction (an α-elimination reaction). The carboxyl group of glycine is released as CO_2 and this comprises the bulk of photorespiratory CO_2 release. The remaining portion of the glycine molecule, the α-carbon and α-amino group, are passed from the PLP group of the P-protein to the lipoic acid group of the H-protein. The lipoic acid group with the bound methylamine physically leaves the active site of the P-protein and moves to the active site of the T-protein. This transferase, the T-protein, transfers the carbon atom of the methylamine group to THF forming N^5,N^{10}-methylene tetrahydrofolate. The amine group, originally the α-amino group of glycine, is released as NH_3. After transfer of the methylamine group, the lipoic group is still carrying the electrons from the oxidation of glycine. This dihydrolipoic acid moves from the active site of the T-protein to the L-protein. A pair of hydrogens are removed from dihydrolipoic acid and the resulting lipoic acid is ready to return to the P-protein to repeat the reaction cycle. FAD is the electron acceptor of the L-protein and those reducing equivalents are passed to NAD^+. The α-carboxyl of glycine is released as CO_2, the α-amino group leaves the mitochondria as NH_3, and the electron pair is transferred to NAD^+.

Support for this reaction mechanism is very strong. All three of the predicted forms of H-protein have been isolated (Neuburger *et al.*, 1991; Walker and Oliver, 1986a). The partial reactions have been demonstrated. The predicted involvement of the different cofactors has been shown in reconstitution experi-

ments and by the use of inhibitors (Sarojini and Oliver, 1985; Walker *et al.*, 1982).

The methylene-THF released from the glycine decarboxylase reaction leaves this complex and serves as a substrate for the serine hydroxymethyltransferase reaction. This PLP-dependent enzyme binds a second molecule of glycine which serves as the methylene acceptor (Turner *et al.*, 1992a). The transfer reaction regenerates the free THF and the three-carbon amino acid, serine (one carbon was the methylene carbon of the first glycine liberated by the GDC reaction and two carbons from the second glycine molecule).

STRUCTURE OF THE GLYCINE DECARBOXYLASE COMPLEX

The glycine decarboxylase complex is structured to support this reaction mechanism (Oliver, 1994). The complex is composed of a core of H-protein subunits. The H-protein does not have a catalytic function but rather the lipoic acid cofactor covalently attached to lys^{63} acts to carry reaction intermediates between the reactive sites of the three larger protein (Neuburger *et al.*, 1991). Thus the H-protein must be physically as well as mechanistically located in the center of the complex. This central core binds the dimers of P-protein and L-protein and the monomers of T-protein. While direct support for this model is limited and will be very hard to obtain as long as we are unable to isolate an intact complex, there is some evidence to support this model. Binary complexes of H-protein and H-protein, H-protein and P-protein, and H-protein and T-protein have been isolated (Neuburger *et al.*, 1991; Walker and Oliver, 1986a), but no complexes between the larger subunits (P-, T-, and L-proteins) have been shown. This supports the idea that the complex is held together by binding to the H-protein. We have been using the dihybrid technique to analyze interactions between the different proteins of the complex as measured by the ability of fusion proteins derived from the component proteins of the complex to interact *in vivo* in yeast (Chien *et al.*, 1991). We are presently using this method to identify amino acids of the H-protein and P-protein that are essential for binding between these two proteins.

The H-protein serves a pivotal role in the function of the complex. The lipoic acid cofactor in its three different forms serves as a substrate for the P-, T-, and L-proteins. In its oxidized form the lipoic acid is a cosubstrate with glycine in the P-protein reaction. The methylamine form of the protein is a cosubstrate with THF for the reaction catalyzed by T-protein. And finally the dihydrolipoamide is a cosubstrate with NAD^+ for the L-protein. When the complex is dissociated into individual proteins, the H-protein must diffuse between the other three proteins and, because it is acting as a substrate, has an apparent K_m value (usually about 2 μM) (Oliver *et al.*, 1990). When the complex reassociates, the rate of the reaction increases as the diffusion path of the lipoic acid is shortened and H-protein no longer shows substrate kinetics. This property along with the tendency of the complex to dissociate at low protein concentrations accounts for much of the difficulty first encountered with working with GDC extracted from mitochondria. The dilution of the mitochondrial matrix when the organelles were disrupted caused the complex to dissociate. Under these conditions, the amount of H-protein was too low to provide measurable rates of glycine oxidation.

METABOLIC REGULATION

Glycine decarboxylase is inhibited by two of its products, serine (Oliver and Sarojini, 1987) and NADH (Bourguignon *et al.*, 1988; Neuburger *et al.*, 1986). Serine binds to the P-protein in a manner that is competitive with the binding of glycine (K_m glycine = 6 mM; K_i serine = 4 mM). NADH binds to the L-protein and its binding is competitive with NAD^+ (K_m NAD^+ = 75 μM; K_i NADH = 15 μM). While it might not be unreasonable to propose feedback control on the complex where rates of serine conversion into 3-phosphoglycerate and its consumption by the C_3 cycle in chloroplasts as well as NADH oxidation by the mitochondrial electron transport chain work together to regulate the rate of glycine oxidation, there is little or no data suggesting that such feedback control occurs *in vivo*. While negative data seldom proves a conjecture, measurements of the rates of glycine oxidation by the isolated enzyme, mitochondria, and leaf discs suggest that the maximum rate of GDC activity is very close to the enzymatic rate needed to maintain observed rates of photorespiration. If there is no excess of glycine decarboxylation capacity, it is difficult to envision the need for a mechanism to slow down the reaction. In fact, most authors (see, for example, Lorimer and Andrews, 1981) have concluded that the only control step in the photorespiratory C_2 cycle is at the level of competition between O_2 and CO_2 for binding to Rubsico and that once phosphoglycolate is formed, the

cycle works to convert all the carbon diverted out of the C3 cycle back to photosynthesis as rapidly as possible (for an alternative explanation see Hanson and Peterson, 1985).

The potential inhibition by NADH and serine is prevented by specialized substrate transporters found in leaf mitochondria (Oliver and McIntosh, 1995). NADH produced by glycine decarboxylation is used to reduce oxaloacetate to malate which can rapidly leave the mitochondria and be oxidized in the cytoplasm or peroxisome to produce NADH and oxaloacetate. Rapid transport rates are possible because of a plant-specific, high-capacity, high-affinity oxaloacetate transporter in the inner mitochondrial membrane (Oliver and Walker, 1984; Ebbighausen *et al.* 1985). Under steady-state photosynthesis a substantial amount of these reducing equivalents are used to reduce hydroxypyruvate to glycerate in the peroxisome (an essential reaction in the C_2 cycle). Serine is exchanged for glycine by means of a glycine:serine antiport found in leaf mitochondria. This combined with a glycine:H^+ symporter keep the glycine/serine ratio favorable for maximal GDC activity (Walker and Oliver 1982; Oliver, 1987).

MUTANTS

The glycine decarboxylase complex has provided a surprisingly flexible experimental system. Somerville and Ogren (1982) were among the first to realize that glycine decarboxylase, like most photorespiratory enzymes, is not essential under nonphotorespiratory conditions. As a result they were able to screen for a number of glycine decarboxylase mutants by selecting for mutagenized *Arabidopsis thaliana* plants that are unable to grow under photorespiratory conditions but could grow normally when photorespiration was inhibited. Normal atmospheric conditions provide high photorespiratory rates and the plant will not grow. High CO_2 concentrations (usually 1000 to 1500 ppm) repress photorespiration and will allow normal growth by the glycine decarboxylase mutants. It is thus possible to grow glycine decarboxylase mutants to maturity. Two GDC loci have been identified in *Arabidopsis* (Somerville and Ogren, 1982) and one locus has been tagged in barley (Blackwell *et al.*, 1990). While the molecular details of these mutations are unknown, one of the *Arabidopsis* mutants lacks P-protein and the barley mutant lacks both P-protein and H-protein. The barley mutation may involve a regulatory element that controls the expression of both proteins or it may be that the mutation prevents the expression of one protein and the lack of this protein prevents the accumulation of the other. While the latter is not uncommon for enzyme complexes, accumulation of partial GDC complexes has been noted in some plants (Rawsthorne, 1992).

The mutational work does suggest that there is no essential nonphotorespiratory function for GDC. In animal tissue, GDC has an essential function and mutants in the complex result in a disease called nonketotic hyperglycinemia (Kume *et al.*, 1988). This genetic disease results in fatal damage to the neurological tissues, probably because of the sensitivity of these tissues to elevated glycine levels. In nonphotorespiratory plant tissues, the complex has three potential functions, the production of glycine from serine, the production of serine from glycine, and the production of C1 fragments in the form of N^5,N^{10}-methylene tetrahydrofolate for biosynthetic functions. The reaction of the complex is readily reversible both *in vitro* and in isolated mitochondria and each of these reactions can be readily identified. Serine can be synthesized from three-carbon intermediates of glycolysis and any serine normally supplied by GDC in the dark could be supplied by this route in GDC mutants. While it is possible for the enzyme to synthesize glycine from CO_2, NH_3, and methylene THF, the rate is rather slow (at least *in vitro*). Glycine can be readily synthesized by reversing the serine hydroxymethyltransferase reaction (serine + THF $\rightarrow$ glycine + methylene-THF) and this enzyme probably compensates for any missing GDC activity. This reaction also produces methylene THF and can, therefore, also provide this function of GDC. At least two forms of SHMT have been identified, a mitochondria isoform that is preferentially expressed in leaf tissues and is involved in photorespiration and a second isoform probably found in the plastid that is expressed more uniformly throughout the plant. Mutations that disrupt the mitochondrial form are not lethal except under photorespiratory conditions, suggesting that the nonmitochondrial form of SHMT has sufficient capacity to supply the glycine and methylene THF needs of the plant.

DEVELOPMENTAL BIOLOGY

While glycine decarboxylase and serine hydroxymethyltransferase are localized within the mitochondria, the very close connections between photorespira-

tion and photosynthesis suggests that these two enzymes may be controlled by the same kinds of mechanism that control the nuclear-encoded photosynthetic enzymes of the chloroplast. This prediction has repeatedly been shown to be correct. Initial observations were based on the glycine decarboxylase activity of mitochondria isolated from green leaves as opposed to etiolated (dark-grown) tissues. Mitochondria from etiolated pea shoots, like mitochondria from all non-photosynthetic tissues we have studied, have low levels of GDC activity (Arron and Edwards, 1980; Moore *et al.*, 1977). When these plants are transferred to the light, there is a 5 to 6 hour lag before the levels of GDC activity in mitochondria isolated from these plants begins to increase (Walker and Oliver, 1986b). When the tissue is fully green the GDC activity increases about 10-fold. The isolation of the component proteins of GDC and the production of antibodies against them showed that this increase in activity resulted from the *de novo* synthesis of these components. Studies using protein synthesis inhibitors revealed that all four of the component proteins were nuclear-encoded and synthesized off 80S cytosolic ribosomes (Walker and Oliver, 1986b).

The increased synthesis of these component proteins in the light results from an increased steady-state level in the mRNA for the proteins. Run-on transcription experiments with isolated pea nuclei showed that this increase in mRNA was controlled at the transcriptional level for the P-protein and H-protein (Srinivasan *et al.*, 1993). During the greening of etiolated peas, the system that has received the most study, the increase in run-on transcription, mRNA level, and protein level are all in the range of 8- to 10-fold (Srinivasan *et al.*, 1992, 1993). Thus, control of expression of P-protein and H-protein during the greening of pea leaves is almost exclusively transcriptional.

We have focused much of our attention on comparing the control of the expression of the H-protein gene (*gdc*H) with the gene for the small subunit of Rubisco (*rbc*S). The level of mRNA for both the *gdc*H and *rbc*S is low in etiolated pea plants and much higher in green leaf tissue (Kim and Oliver, 1990; Macherel *et al.*, 1990; Turner *et al.*, 1993; Srinivasan and Oliver, 1992). It thus appears that the increase in both proteins during greening of etiolated tissues is controlled at the level of mRNA concentration. By comparing the kinetics of mRNA accumulation for both *gdc*H and *rbc*S, it should be possible to determine if they are regulated by similar transcriptional mechanisms. Following illumination of these etiolated seedlings, there is a 4 to 5 hour delay before the level of *gdc*H and *rbc*S mRNA begins to increase (Srinivasan *et al.*, 1992, 1993).

The reason for the 4 to 5 hour lag before mRNA levels begin to increase is thought to be the time needed to develop mature chloroplast (Susek and Chory, 1992; Taylor, 1989). The mature chloroplasts somehow signal the nucleus and cause maximum expression of both genes. There are two lines of evidence that suggest this is happening with the expression of H-protein. When the greening experiments are repeated with light-grown pea plants that had been transferred to the dark for 72 hours, the kinetics of H-protein mRNA accumulation change substantially. The level of *gdc*H as well as *rbc*S mRNA in these dark-adapted green plants is much lower than in etiolated plants. When the plants are transferred to the light, the lag time for the accumulation of both mRNA species decreases from 4 or 5 hours to about 1 hour and the rate of accumulation is much higher (Srinivasan *et al.*, 1992, 1993). These plants have mature chloroplasts and, therefore, these nuclear genes are activated immediately without the time lag needed for plastid maturation. The second line of evidence comes from the use of the herbicide, Norflurazon, which blocks the synthesis of carotenoids. The lack of carotenoids results in photobleaching in strong light and damage to the chloroplasts. When peas are grown in increasing light intensity the amount of H-protein mRNA increases in a standard light saturation curve with a half-maximum H-protein mRNA accumulating at 10 $\mu E/m^2/sec$. When the plants are grown in the presence of Norflurazon, the level of H-protein mRNA increases to a light intensity of 5 to 10 $\mu E/m^2/sec$ in parallel with the increase in chlorophyll accumulating in the plants. At higher light intensities photobleaching causes a loss of chlorophyll and no H-protein mRNA accumulates. In bright light the Norflurazon blocks the formation of mature chloroplasts and the resulting lack of a signal precludes expression of the H-protein mRNA (Srinivasan *et al.*, 1993).

Turner *et al.* (1993) have done a careful analysis of the accumulation of the mRNA and protein for all four subunits of GDC as well as SHMT during the greening of etiolated pea plants. This analysis showed a strong correlation between the amount of mRNA and the amount of protein during the greening process. L-protein was the exception because there was a substantial amount in etiolated leaves due to its role in other multienzyme complexes. They concluded that the balance between the different subunits of the com-

plex is maintained by differential rates of gene transcription.

In order to better understand the mechanism by which light controls the expression of the gene coding for H-protein, *gdcH*, we have isolated the genomic clone for this gene from *Arabidopsis*, fused the promoter for that gene to the reporter gene β-glucuronidase (GUS), and transformed this construct into tobacco. Comparisons of the expression of the levels of GUS expression with those of the endogenous H-protein showed that the clone containing 856 bp of promoter and 62 bp of 5′ untranslated region contained all the information needed for light-dependent and tissue-specific expression of the reporter gene (Fig. 2). The *gdcH*:GUS construct expressed 6.5 times more GUS activity in light-grown as opposed to dark-grown tissue and 17 times more GUS activity in leaves compared to roots (Srinivasan and Oliver, unpublished).

A preliminary deletion analysis of the promoter showed that most of the light dependence and all of the tissue specificity was conferred by a 259 bp fragment between −376 bp and −117 bp (Fig. 2). Sequence analysis suggested several putative GT boxes within this sequence (Gilmartin *et al.*, 1990). Gel retardation experiments using nuclear extracts from tobacco leaf and root nuclei demonstrate specific binding of one or more nuclear factors to this fragment (Fig. 3). These protein factors are specific for this 259 bp fragment and are found in nuclei from both green and etiolated leaves. The factors are less prevalent in root nuclei (Raman and Oliver, unpublished).

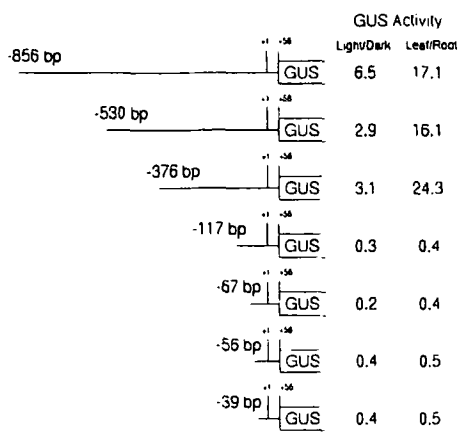

Fig. 2. Expression of *gdcH*:GUS fusion in transgenic tobacco. Different length *gdcH* promoters were fused to the GUS structural gene and transformed into tobacco. Seeds from regenerated plants were planted and grown in the light and dark. The ratio of activity in light-grown/dark-grown plants and the leaf/root activity in light-grown plants is reported for each of the different constructs.

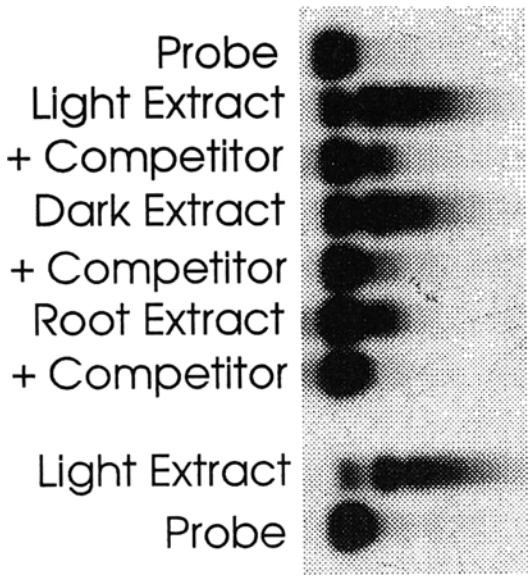

Fig. 3. Gel shift analysis of the 259 bp fragment identified as responsible for light-dependent and tissue-specific expression of *gdcH*. The 259 bp fragment between −117 bp and −356 bp was labeled and used in gel shift experiments employing protein extracts from nuclei isolated from green tobacco leaves, etiolated tobacco leaves, and tobacco roots. The specific competitor was a 30-fold excess of the unlabeled fragment.

Interestingly, once the strong light-responsive elements downstream of −117 bp are deleted, the *gdcH*:GUS constructs are more strongly expressed in the dark than in the light and in the root compared to the leaves (Fig. 2). This may suggest a weak element near the transcriptional start site that is responsible for low-level expression of the gene in the dark and in nonphotosynthetic tissue. Once the stronger upstream light-responsive elements are removed this relatively weak (<5% of the full length promoter) dark-enhanced element becomes perceptible. The 5′ upstream untranslated region contains a ACAAAA sequence. This element was originally identified as an enhancer in the ferredoxin gene and is found in several light-dependent genes (Casper and Quail, 1993).

SUMMARY

The glycine decarboxylase complex offers an unusually rich experimental system for studying a number of problems in contemporary plant biology. It is an abundant protein encoded by four single copy genes (at least in *Arabidopsis*). The expression of the genes is under temporal and spatial control. By chang-

ing the plant growth conditions (specifically CO_2 and probably O_2 concentration) one can readily make the complex either essential or redundant. It is a mitochondrial protein and represents one of the very few developmental systems known in plant mitochondria. The complex is made up of four different subunits which can readily dissociate and reassociate *in vitro,* thus allowing studies on subunit structure and protein:protein interactions. In several ways it is one of the more versatile model systems available in higher plants.

REFERENCES

Arron, G. P., and Edwards, G. E. (1980). *Plant. Sci. Lett.* **18**, 229–235.

Blackwell, D., Murray, A. J. S., and Lea, P. J. (1990). *Plant Physiol.* **94**, 1316–1322.

Bourguignon, J., Neuburger, M., and Douce, R. (1988). *Biochem. J.* **255**, 169–178.

Bourguignon, J., Macherel, D., Neuburger, M., and Douce, R. (1992). *Eur. J. Biochem.* **204**, 865–873.

Bourguignon, J., Vauclare, P., Merand, V., Forest, E., Neuburger, M., and Douce, R. (1993). *Eur. J. Biochem.* **217**, 377–386.

Bowes, G., Ogren, W. L., and Hageman, R. H. (1971). *Biochem. Biophys. Res. Commun.* **45**, 716–722.

Camp, P. J., and Randall, D. D. (1985). *Plant Physiol.* **77**, 571–577.

Casper, T., and Quail, P. H. (1993). **3**, 161–174.

Chien, C.-T., Bartel, P. L., Sternglanz, R., and Fields, S. (1991). *Proc. Natl. Acad. Sci. USA* **88**, 9578–9582.

Decker, J. P. (1955). *Plant Physiol.* **30**, 82–89.

Ebbighausen, H., Jia, C., and Heldt, H. W. (1985). *Biochim. Biophys. Acta* **47**, 184–199.

Fujiwara, K., Okamura-Ikeda, K., and Motokawa, Y. (1990). *J. Biol. Chem.* **265**, 17463–17467.

Fujiwara, K., Okamura-Ikeda, K., and Motokawa, Y. (1992). *J. Biol. Chem.* **267**, 20011–20016.

Gilmartin, P. M., Sarokin, L., Memelink, J., and Chua, N-H. (1990). *Plant Cell* **2**, 369–378.

Hanson, K. H., and Peterson, R. B. (1985). *Arch. Biochem. Biophys.* **237**, 300–313.

Husic, D. W., Husic, H. D., and Tolbert, M. E. (1987). *Crit. Rev. Plant Sci.* **5**, 45–100.

Kikuchi, G. (1973). *Mol. Cell. Biochem.* **1**, 169–187.

Kim, Y., and Oliver, D. J. (1990). *J. Biol. Chem.* **265**, 848–853.

Kim, Y., Shah, K., and Oliver, D. J. (1991). *Physiol. Plant.* **81**, 501–506.

Kopriva, S., and Bauwe, H. (1994). *Plant Physiol.* **104**, 1079–1080.

Kuhlemeier, C., Green, P. J., and Chua, N-H. (1987). *Annu. Rev. Plant Physiol.* **38**, 221–257.

Kume, A., Kure, S., Tada, K., and Hiraga, K. (1988). *Biochem. Biophys. Res. Commun.* **154**, 292–297.

Lorimer, G. H., and Andrews, T. J. (1981). In *The Biochemistry of Plants, A Comprehensive Treatise* (Hatch, M. D., and Boardman, N. K., eds.), Vol. 8, Academic Press, New York, pp. 329–374.

Macherel, D., Lebrun, M., Gagnon, J., Neuburger, M., and Douce, R. (1990). *Biochem. J.* **268**, 783–789.

Moore, A. L., Jackson, C., Halliwell, B., Dench, J. E., and Hall, D. O. (1977). *Biochem. Biophys. Res. Commun.* **78**, 483–491.

Neuburger, M., Bourguignon, J., and Douce, R. (1986). *FEBS Lett.* **207**, 18–22.

Neuburger, M., Jourdain, A., and Douce, R. (1991). *Biochem. J.* **278**, 765–769.

Oliver, D. J. (1994). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 323–337.

Oliver, D. J. (1987). In *Plant Mitochondria: Structural, Functional, and Physiological Aspects* (Moore, A. L., and Beechey, R. B., eds.), Plenum Press, pp. 219–222.

Oliver, D. J., and Kim, Y. (1990). In *Biochemical and Genetic Regulation of Photosynthesis* (Zelitch, I., ed.), Alan R. Liss, Inc., New York, pp. 253–269.

Oliver, D. J., and McIntosh, C. A. (1995). In *The Molecular Biology of Plant Mitochondria* (Levings, C. S., III, and Vasil, I. K., eds.), Kluwer Publishers, The Netherlands, pp. 237–280.

Oliver, D. J., and Sarojini, G. (1987). In *Progress in Photosynthesis Research* (Biggins, J., ed.), Martinus Nijhoff Publishers, The Hague, pp. 573–576.

Oliver, D. J., and Walker, G. H. (1984). *Plant Physiol.* **76**, 409–413.

Oliver, D. J., Neuburger, M., Bourguignon, J., and Douce, R. (1990). *Plant Physiol.* **94**, 833–839.

Pares, S., Cohen-Addad, C., Sieker, L., Neuburger, M., and Douce, R. (1994). *Proc. Natl. Acad. Sci. USA* **91**, 4850–4853.

Rawsthorne, S. (1992). *Plant J.* **2**, 267–274.

Sarojini, G., and Oliver, D. J. (1983). *Plant Physiol.* **72**, 194–199.

Sarojini, G., and Oliver, D. J. (1984). In *Advances in Photosynthesis Research* (Sybesma, C., ed.), Vol. III, Nijhoff/Junk Publishers, The Hague, pp. 553–556.

Sarojini, G., and Oliver, D. J. (1985). *Plant Physiol.* **77**, 786–789.

Sieker, L., Cohen-Addad, C., Neuburger, M., and Douce, R. (1991). *J. Mol. Biol.* **220**, 223–224.

Somerville, C. R., and Ogren, W. L. (1982). *Biochem. J.* **202**, 373–380.

Srinivasan, R., and Oliver, D. J. (1992). *Plant Physiol.* **98**, 1518–1519.

Srinivasan, R., Kraus, C., and Oliver, D. J. (1992). In *Molecular, Biochemical, and Physiological Aspects of Plant Respiration* (Lambers, H., and van der Plas, L. H. W., eds.), SPB Academic Publishing, The Hague, pp. 323–334.

Srinivasan, R., Berndt, W. A., and Oliver, D. J. (1993). In *Plant Mitochondria* (Brennicke, A., and Kuck, U., eds.), VCH, Veinheim, pp. 160–169.

Susek, R. E., and Chory, J. (1992). *Aust. J. Plant Physiol.* **19**, 387–399.

Taylor, W. C. (1989). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 211–233.

Tolbert, N. E. (1979). In *Encyclopedia of Plant Physiology* (Gibbs, M., and Latzko, E., eds.), Vol. 6, Springer-Verlag, Berlin, pp. 338–352.

Turner, S. R., Ireland, R., Morgan, C., and Rawsthorne, S. (1992a). *J. Biol. Chem.* **267**, 13528–13534.

Turner, S. R., Ireland, R., and Rawsthorne, S. (1992b). *J. Biol. Chem.* **267**, 5355–5360.

Turner, S. R., Ireland, R., and Rawsthorne, S. (1992c). *J. Biol. Chem.* **267**, 7745–7750.

Turner, S. R., Hellen, R., Ireland, R., Ellis, N., and Rawsthorne, S. (1993). *Mol. Gen. Genet.* **236**, 402–408.

Walker, G. H., and Oliver, D. J., (1982). *Biochem. Biophys. Res. Commun.* **107**, 856–861.

Walker, G. H., Oliver, D. J., and Sarojini, G. (1982). *Plant Physiol.* **70**, 1465–1469.

Walker, J. L., and Oliver, D. J. (1986a). *J. Biol. Chem.* **261**, 2214–2221.

Walker, J. L., and Oliver, D. J. (1986b). *Arch. Biochem. Biophys.* **248**, 626–638.

Woo, K. C., and Osmond, C. B. (1976). *Aust. J. Plant Physiol.* **3**, 771–785.

Zelitch, I. (1971). *Photosynthesis, Photorespiration, and Plant Productivity,* Academic Press, New York.