A long period of drought leads to stomatal closure, thus depriving most of the green cells of CO₂. Under these conditions, glycine is released from the chloroplasts via phosphoglycolate, from ribulose-1,5-bisphosphate by the oxygenase reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase [1, 2]. The resulting glycine is then transported into the peroxisomes to be metabolized through the oxidative photosynthetic carbon cycle (C₂ cycle). In the peroxisomes, glycine is oxidized by the flavoprotein glycolate oxidase, producing glyoxylate which is transaminated to glycine. Glycine, in turn, migrates to the mitochondria where it is converted to serine, NH₃, and CO₂. Serine then returns to the peroxisomes where, via the action of a transaminase and hydroxypyruvate reductase, glyceraldehyde is formed. Finally, glyceraldehyde enters the chloroplast where it is phosphorylated by glyceraldehyde kinase to give glyceraldehyde 3-phosphate, to regenerate ribulose-1,5-bisphosphate after reduction to triose-phosphate via the Calvin-Benson cycle (C₃ cycle) [1, 2]. During photosynthesis in higher plants, no net synthesis of non-photosynthetic carbohydrates occurs; the light energy is used to drive the utilization (through the C₂ cycle) and the generation (through the C₃ cycle) of ribulose-1,5-bisphosphate, thus preventing the formation of the excited triplet state of chlorophyll and excess reactive O₂ species (superoxide radicals and singlet oxygen). Generally, the reactions of the C₂ cycle are not believed to be regulated after the site of carbon entry (phosphoglycolate synthesis by ribulose-1,5-bisphosphate carboxylase/oxygenase) into the pathway. The conversion of glycine to serine in green-leaf mitochondria is currently considered to be the major source of CO₂ released during photorespiration [1, 2]. In this short review, we will consider only the mechanisms of glycine oxidation in green-leaf mitochondria.

Subunit structure and reaction mechanism

During the course of the C₂ cycle, glycine molecules formed in the peroxisomes are immediately broken down by a complex of proteins (glycine decarboxylase or glycine cleavage) localized in the mitochondrial matrix [3], which, by concerted their activities, catalyse the oxidative decarboxylation and deamination of glycine with the formation of CO₂, NH₃ and N₅,N¹⁰-methylene-5,6,7,8-tetrahydrofolate (CH₂H₄PteGlu, with n glutamate residues) [1, 2]. The latter compound produced reacts with a second molecule of glycine to form serine and 5,6,7,8-tetrahydrofolate (H₄PteGlu, in a reaction catalysed by serine hydroxymethyltransferase. The glutamate chain length (one to six glutamate residues) influences the affinity constant for H₄PteGlu, and the maximal velocities displayed by these two enzymic systems. Indeed, the affinity constant decreases by at least one order of magnitude when the tetrahydrofolate substrate contains three or more glutamate residues [4]. Analyses of pea-leaf mitochondrial folate reveal a pool of polyglutamates dominated by tetra- and penta-glutamates [4].

Glycine decarboxylase has been purified from plant mitochondria [5, 6], animals [7, 8], and bacteria [9, 10] and consists of four protein components which have been tentatively named 'P-protein' (a homodimer of 97 kDa peptides containing pyridoxal phosphate), 'H-protein' (a 14.2 kDa lipoamide-containing protein), 'T-protein' (a homodimer of 60 kDa peptides containing FAD) and 'L-protein' (a homodimer of 60 kDa peptides containing FAD) (Figure 1). The glycine decarboxylase complex from plant-leaf mitochondria is closely related to similar enzyme complexes found in bacteria such as 

*Petrococcus glycinophilus* [9] and *Athrobacter globiformis* [10], and in the mitochondria of animal tissues [7]. Parenthetically, the dihydrolipoamide dehydrogenase component of the glycine decarboxylase complex, which contains, in addition to FAD, a redox-active cysteine residue, is also a component (called E₂) of a family of multi-enzyme complexes that is composed of pyruvate, 2-oxoglutarate, and branched-chain 2-oxoacid dehydrogenases [11]. The H-protein provides the attachment site for the lipoic acid (attached via a amide linkage to the ε-amino group of a lysine residue, Lys-63 on the pea H-protein) which interacts in a very flexible manner with each of the other three proteins (P-, T- and L-proteins) in the multi-

Abbreviations used: CH₃H₄PteGlu,, N₅,N¹⁰-methylene-5,6,7,8-tetrahydrofolate (CH₂H₄PteGlu, with n glutamate residues); H₄PteGlu, 5,6,7,8-tetrahydrofolate; OAA, oxaloacetate; SHMT, serine hydroxymethyltransferase.
Figure 1
Scheme outlining the reactions involved in oxidative decarboxylation and deamination of glycine in plant mitochondrial matrix

P, H, T and L are the protein components of the glycine cleavage (glycine decarboxylase) system. SHMT is involved in the recycling of CH$_3$H,F,Glu, with n glutamate residues into H,F,Glu.

**enzyme complex.** During the early steps of H-protein isolation, when P- and H-protein react together in the presence of glycine the methylamine intermediate (bound to the lipoamide of the H-protein) accumulates in the medium at the expense of oxidized H-protein [12]. Under these conditions, the methylamine intermediate, which is a rather stable structure, is easily separated from the oxidized H-protein on ion-exchange chromatography [12]. All of the reactions catalysed by the glycine cleavage system are fully reversible. For example, the H- and L-proteins together catalyse the reversible exchange of electrons between NADH and lipoamide bound to the H-protein [12].

The equilibrium constant of the serine hydroxymethyltransferase suggests that, during photorespiration, the reaction must be permanently pushed toward the formation of serine (the unfavourable direction) to allow the recycling of H,F,PteGlu, necessary for the operation of the T-protein component of the glycine decarboxylase system (Figure 1). Serine hydroxymethyltransferase (SHMT) also requires pyridoxal phosphate and, in its native form, is a 220 kDa tetramer composed of four identical subunits, each with a molecular mass of 53 kDa [6]. Each subunit has a pyridoxal phosphate bound as a Schiff base to an ε-amino group of lysyl residue.

The rate of glycine release during the course of photorespiration is as much as 50% of the photosynthetic rate of about 3 μmol CO$_2$ fixed/mg chlorophyll per min and some ten times the rate of normal tricarboxylic acid cycle activity. In order to accomplish rapid rates of glycine oxidation, to cope with all the glycine molecules flooding out of the peroxisomes, the glycine cleavage system linked to SHMT is present at tremendously high concentrations within the mitochondrial matrix (where it comprises about half of the soluble proteins in mitochondria from fully expanded green leaves) [13]. This is in contrast with the situation observed in mammalian mitochondria where glycine decarboxylase represents a minute fraction of the total matrix protein [14]. This high protein concentration (0.2 g/ml) influences biorecognition processes and leads to the formation of a loose multi-enzyme complex (approximate subunit ratio of 2 P-protein dimers: 27 H-protein monomers: 9 T-protein monomers: 1 L-protein dimer) [13] with enzymological properties that are very different from those of the dissociated form of the complex which occurs at low protein concentrations [15]. Indeed, when the
complex is diluted, it tends to dissociate into its component enzymes. In the dissociated state, the H-protein acts as a mobile cosubstrate that commutes between the other three enzymes and shows typical substrate kinetics. On the other hand, when the complex is reformed, the H-protein no longer acts as a substrate but as an integrated part of the enzyme complex [15].

**Developmental control**

cDNA clones encoding P-, H-, T- and L-proteins of the glycine cleavage system of pea mitochondria have been isolated and characterized [16-21]. The deduced primary structure revealed that all the polypeptides were synthesized in the cytoplasm with a mitochondrial targeting peptide. In addition, all four proteins of the complex are encoded by unique nuclear genes, translated on cytosolic ribosomes, and imported into the mitochondrial matrix [16-21]. The glycine decarboxylase is present in low amounts in etiolated pea leaves, but increases considerably upon exposure to light [16-21]. In fact, expression of the genes encoding H-, P- and T-proteins was shown to occur specifically in the mature leaf tissue, with light exerting an additional effect by increasing the mRNA levels several-fold.

**Figure 2**

Schematic representation of glycine oxidation in green-leaf mitochondria

During photorespiration glycine is cleaved in the matrix space by the glycine cleavage system (see Figure 1) to CO₂, NADH, and CH₂H₂Figu,. The latter compound produced reacts with a second molecule of glycine to form serine and H₂Figu, in a reaction catalysed by SHMT. NADH produced during the course of glycine oxidation is oxidized, either by the respiratory chain or by OAA, owing to the malate dehydrogenase located in the matrix space working in the reverse direction. A rapid malate-OAA transport shuttle appears to play an important role in the photorespiratory cycle, in catalysing the transfer of reducing equivalents generated in the mitochondria during glycine oxidation to the peroxisomal compartment for the reduction of β-hydroxypyruvate. Note the unusual stoichiometry of two glycine molecules entering the mitochondrial matrix in exchange for one serine leaving. A consideration to be taken into account, regarding the possibility of glycine oxidation in vivo via the respiratory chain, is that this process necessarily leads to the production of ATP, which must be recycled back as ADP. Abbreviations used: GDC, glycine decarboxylase; im, inner membrane; om, outer membrane; CH₂H₂Figu,; CH₂H₂PteGlu,; H₂Figu,; H₂PteGlu,.
[16–22]. However, Northern blot analysis of RNA from different tissues, such as leaf, root and stem, using L-protein cDNA as a probe, indicates that the mRNA of the dihydrolipoamide dehydrogenase accumulates to identical levels in all tissues [19, 20]. Such a result is expected because in higher plants the mitochondrial enzyme complexes, glycine decarboxylase, pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, all use the same lipoamide dehydrogenase complex [19–20]. Macherel et al. [23] have isolated and characterized the gene encoding the H-protein in pea. The H-protein gene contains three introns located in the coding region and two main transcription sites detected by primer extension of RNA. The presence of two potential promoters may be related to the specialized overexpression pattern of H-protein in leaves.

**Metabolic control**

Apparently glycine cleavage activity is not affected by light, by reversible covalent modification, by control proteins or by proteolytic activation. Its catalytic activity is only regulated by the NADH/NAD+ molar ratio: with NAD’ and NADH acting competitively with respect to NAD+, K,,(NAD+) = 75 μM; K,(NADH) = 15 μM) [3]. Likewise, serine inhibits the reaction competitively with glycine [K,(serine) = 4 mM; K,(glycine) = 6 mM] and appears to bind to the P-protein [24]. NADH generated in the matrix space during the course of glycine oxidation must therefore be re-oxidized if the photorespiratory cycle is to continue. NADH produced in vivo is re-oxidized very rapidly by oxaloacetate (OAA), owing to the tremendous excess of malate dehydrogenase located in the matrix space working in the reverse direction. Indeed, a very powerful phthalonate-sensitive OAA carrier has recently been characterized in all the plant mitochondria isolated so far [K,,(OAA) = 5 μM; V,, = 700 nmol/mg protein per min] [25, 26]. This rapid malate–OAA transport shuttle, the equivalent of which is not found in mammalian mitochondria, appears to play an important role in the photorespiratory cycle, catalysing the transfer of reducing equivalents generated in the mitochondria during glycine oxidation to the peroxisomal compartment for the reduction of β-hydroxybutyrate [1, 2] (Figure 2). In the presence of OAA, the glycine cleavage is functioning at maximum capability, and at a significantly faster rate than when it is supported only by NADH reoxidation via Complex I under state 3 conditions [27]. In green leaves, the rapid reoxidation of NADH and the immediate utilization of NH₃ (via glutamate synthase and glutamine synthetase operating in a concerted manner) and CO₂ (via ribulose-1,5-bisphosphate carboxylase) during the course of glycine oxidation continuously shift the equilibrium toward serine formation even though the reactions are readily reversible in vitro.

Finally, compounds that react with either the lipoamide cofactor of the H-protein (such as arsenite) or with the pyridoxal phosphate of the P-protein (such as carboxymethoxylamine, methoxylamine and acetylhydrazide), strongly inhibit the glycine cleavage system [28]. We believe that molecules acting at the level of the protein components of the glycine decarboxylase complex would exhibit herbicidal potency. Indeed, experiments using Arabidopsis thaliana showed that in plants deficient in glycine decarboxylase activity, photosynthesis was not impaired in non-photorespiratory conditions, but was irreversibly inhibited in atmospheres that allowed the rapid production of glycolate by chloroplasts [29, 30].
Protease maturation of the Rieske iron–sulphur protein after its insertion into the mitochondrial cytochrome bc₁ complex of Saccharomyces cerevisiae
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The iron–sulphur protein of the mitochondrial cytochrome bc₁ complex is encoded on the nuclear genome, synthesized on cytoplasmic polysomes, and imported post-translationally into the organelle, where it is inserted into the cytochrome bc₁ complex in the inner mitochondrial membrane [1, 2]. At some point during import and assembly, a mitochondrial targeting presequence is removed from the precursor form of iron–sulphur protein (p-ISP) by mitochondrial protease(s), and a 2Fe–2S cluster is inserted into the apoprotein. We have been using a genetic and molecular biological approach to elucidate the details of import and assembly of the iron–sulphur protein in Saccharomyces cerevisiae [3–5].

In Neurospora crassa [6] and S. cerevisiae [7], post-translational protease processing of the iron–sulphur protein occurs in two steps. Initially a mitochondrial matrix protease (MPP) removes 22 amino acids from p-ISP to generate intermediate iron–sulphur protein (i-ISP). This proteolytic processing is then followed by a second proteolytic cleavage, catalysed by a mitochondrial intermediate protease (MIP), which removes an octapeptide from i-ISP to generate mature-sized iron–sulphur protein (m-ISP). It is generally agreed that MPP is located in the mitochondrial matrix, but the location of MIP remains to be established. It also remains to be established whether there is one or more MIPs which remove octapeptides from the multiple nuclear-encoded mitochondrial proteins which are processed in two steps [8, 9].

The amino acid sequence and predicted secondary structure of the iron–sulphur protein of the yeast cytochrome bc₁ complex are shown in Figure 1. The MPP and MIP protease cleavage sites are indicated by scissors. The full-length precursor protein consists of 215 amino acids. After two-step protease processing, Lys-31 resides at the N-terminus of m-ISP. Figure 1 also shows the location of multiple temperature-sensitive and petite alleles of the iron–sulphur protein which were generated by random mutagenesis of the cloned gene [10]. Two aspects of these mutant loci are noteworthy. Firstly, the mutations are clustered in the C-terminal portion of the protein, and most of these are located in what is predicted to be the internal ‘core’ surround-