Molecular Cloning and Characterization of Complementary DNA Encoding for Ferredoxin-dependent Glutamate Synthase in Maize Leaf*

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The sequence of ferredoxin-dependent glutamate synthase (EC 1.4.7.1) mRNA from maize has been determined. Complementary DNAs were isolated from a cDNA library of light-induced leaf poly(A)* RNA constructed in an expression vector. An open reading frame beginning at an ATG codon at nucleotide 328 of the longest cDNA (5617-bases long) encoded 1616 amino acid residues. The amino terminus of the purified mature enzyme coincided with the cysteine residue at position 98 of the predicted sequence. This enzyme is homologous with the large subunit of Escherichia coli NADPH-dependent glutamate synthase having about 42% identical residues between the two proteins. The enzyme also contains a short region similar to a potential FMN-binding region of yeast flavocytochrome b. The cDNA hybridizes to an RNA band about 5.5 kilobases whose steady-state level is markedly increased upon illumination of etiolated maize seedlings. Analysis of genomic DNA indicates the presence of a single-copy gene for ferredoxin glutamate synthase in maize.

In most plants, glutamine synthetase and glutamate synthase (GOGAT)* are key enzymes involved in the assimilation of ammonia which is derived from both external nitrogen sources and internal nitrogen metabolic processes such as photorespiration and amino acid catabolism. Glutamine synthetase catalyzes the initial incorporation of free ammonia into glutamine using glutamate as an acceptor, and GOGAT catalyzes the transamidation of the amido nitrogen from glutamine to 2-oxoglutarate to form two molecules of glutamate. The two enzymes are thus dependent on each other for the supply of their substrates, composing a metabolic route called the glutamine synthetase/GOGAT cycle. The importance of this cycle for the general nitrogen utilization in plant cells is well documented (Miflin and Lea, 1980).

GOGAT exists in higher plants in two forms, each of which requires a specific electron donor for the reaction; one utilizes ferredoxin (Fd) as a reductant (glutamate synthase (ferredoxin), EC 1.4.7.1) and the other utilizes NAD(P)H (glutamate synthase (NADH), EC 1.4.1.14; glutamate synthase (NADPH), EC 1.4.1.13) (Suzuki and Gadal, 1984). In addition to the reductant specificity, the two enzymes differ in molecular size, kinetics, and antigenicity, indicating that they are structurally distinct proteins (Suzuki and Gadal, 1984).

Fd-GOGAT, rather than the pyridine nucleotide-dependent form, is the major component in green tissues (Matoh and Takahashi, 1982; Wallsgrove et al., 1982) and is localized in the chloroplasts (Lea and Miflin, 1974; Botella et al., 1988). It has been shown that Fd-GOGAT is essential for the reassociation of photosynthetic ammonia in green leaves by studying photorespiratory mutants (Somerville and Ogen, 1990; Kendall et al., 1986). The enzyme is also known to exist in non-photosynthetic tissues such as roots (Suzuki et al., 1982) and nodules (Suzuki et al., 1984a), although its physiological role is not fully understood (Suzuki et al., 1984b; Oaks and Hirel, 1985). According to recent reports (Hirasawa and Tamura, 1984; Marquez et al., 1988), Fd-GOGAT from green leaves is a monomeric protein with a molecular mass ranging from 160 to 140 kDa and contains iron-sulfur cluster and flavins (FAD and FMN), although several previous reports suggested the absence of the prosthetic groups (Wallsgrove et al., 1977; Tamura et al., 1980; Suzuki and Gadal, 1982). NADH-GOGAT has been reported in roots (Suzuki et al., 1984b), shoots (Matoh et al., 1980; Suzuki et al., 1982), and nodules (Groat and Vance, 1981; Chen and Cullimore, 1988; Anderson et al., 1988). It appears to be an iron-sulfur flavoprotein composed of a single polypeptide of molecular mass between 200-240 kDa. The two types of GOGAT show different developmental patterns in early growth stages of plant seedlings (Matoh and Takahashi, 1982), and their expressions are influenced by environmental factors such as light and exogenous nitrogen sources (Watanabe et al., 1985; Hecht et al., 1988). Fd-GOGAT shows low activity in young or etiolated shoots but increases rapidly upon plant development or greening, whereas NADH-GOGAT is not changed, or gradually decreases, during these periods to become only a minor component in mature green leaves.

The coexistence of Fd-GOGAT and NAD(P)H-GOGAT is a general phenomenon in higher plants, and probably is related to different physiological functions of the enzymes. The expression of the two forms seems to be under different regulatory controls. However, molecular tools have been lacking to study more extensively the structural characteristics of GOGAT proteins and regulatory mechanisms for gene expression. We have focused on the cDNA cloning of maize Fd-GOGAT in order to elucidate the complete primary structure of the protein and obtain a nucleic acid probe for further genetic studies.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05739.

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‡ The abbreviations and trivial names used are: GOGAT, glutamate synthase; PMSF, phenylmethylsulfonyl fluoride; TBS, Tris-buffered saline; kb, kilobase; SDS, sodium dodecyl sulfate; bp, base pair.
Many bacterial species are known to have a pyridine nucleotide-dependent GOGAT and the enzyme structure has been extensively studied. The enzymes from Escherichia coli (Miller and Stadtman, 1972), Bacillus megaterium (Hemmila and Mantsala, 1978), and Aerobacter aerogenes (Trotta et al., 1974) are composed of two nonidentical subunits, a large and a small one, whose estimated molecular masses vary from 135 to 175 kDa and from 51 to 55 kDa, respectively. The enzymes have been extensively studied. The enzymes from Escherichia coli that of Fd-GOGAT (Miniprint), and 3) Fd-GOGAT had complementarity with the 88-kDa fragment (Miniprint). 2) A sum of amino acid composition of the two fragments agreed reasonably with the amino acid composition of Fd-GOGAT (Fig. 1). The two polypeptides were not present in the original extract and were demonstrated to be derived from different regions of Fd-GOGAT, most probably by a limited cleavage of the Fd-GOGAT polypeptide based on the following observations. 1) The NH$_2$-terminal sequence of Fd-GOGAT was identical with that of 73-kDa fragment but not with the 88-kDa fragment (Miniprint). 2) A sum of amino acid composition of the two fragments agreed reasonably with that of Fd-GOGAT (Miniprint), and 3) Fd-GOGAT had common antigenicity with the two fragments, whereas the two were antigenically different from each other (Miniprint). Collectively, these data suggest that the 73- and 88-kDa fragments are derived from the NH$_2$- and COOH-terminal sides of Fd-GOGAT, respectively. As described below, it was confirmed that the NH$_2$-terminal sequence of the 88-kDa fragment is found at the internal region of a polypeptide deduced from the nucleotide sequence of a cDNA encoding maize Fd-GOGAT.

The appearance of the nicked enzyme is likely to be artificial due to a proteolytic cleavage during dialysis. These cleaved fragments were not found in a freshly prepared leaf extract. Removal of the reducing reagent 2-mercaptoethanol from the dialysis buffer resulted in a partial protection of the cleavage (data not shown).

**Isolation and Characterization of Fd-GOGAT cDNA**—Since the apparent molecular mass of maize Fd-GOGAT was approximately 160 kDa as determined by SDS-polyacrylamide gel electrophoresis, the length of mRNA required to encode Fd-GOGAT was estimated to be at least 4.8 kb. To increase the efficiency of cloning a cDNA encoding Fd-GOGAT, our preparation of cDNA synthesized with maize poly(A)$^+$ RNA was size-fractionated by gel filtration and the resulting cDNA molecules ranging from 1.5 to 7 kb were ligated into the pUEX-1 vector. The cDNA library thus constructed was screened by digestion of the insert DNA with AluI, HaeIII, Sau3AI, and TaqI were used for nucleotide sequencing. About 90% of the total structure was determined on both strands and there was no ambiguity in the remaining region, which was sequenced in one direction only. The nucleotide sequence

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**Fig. 1.** Detection of maize Fd-GOGAT and its related polypeptides by Western blotting. A crude extract of green maize leaves (lane 1) and the dialysate of a 35-70% ammonium sulfate precipitate (lane 2) were separated by SDS-polyacrylamide gel electrophoresis. The gel was stained with Coomassie Brilliant Blue (A) or blotted to PVDF membrane followed by immunodecoration using antibodies against maize Fd-GOGAT (B). Migration of molecular size markers are shown on the left side; phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), and carbonic anhydrase (30 kDa). A band with the lowest mobility detected in lane 2 of panel B may be derived from an aggregation of Fd-GOGAT polypeptide.

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**Fig. 2.** Restriction maps and sequence strategies of pFDGGT11 and pFDGGT53. Only relevant restriction sites are indicated, and both clones showed the same restriction map. Fragments obtained by various endonucleases were subcloned into M13 vectors, and their nucleotide sequences were determined. Arrows indicate the direction and extent of sequence determined. The coding region is represented by an open bar. Positions of probe I and probe II used for further studies are indicated above the open bar of pFDGGT11.
Fig. 3. Nucleotide sequence of cDNA and amino acid sequence of Fd-GOGAT. As the nucleotide sequences of pFDGGT11 and pFDGGT53 were overlapped as shown in Fig. 2, the two are combined to give one sequence of cDNA for Fd-GOGAT. The sequence of pFDGGT11 starts at nucleotide 506 and ends at nucleotide 5195 where polyadenylation occurs. The lines below the amino acid sequence indicate the NH2-terminal sequences of the authentic Fd-GOGAT (line a) and 88-kDa fragment (line b), which were determined by protein sequencing.
essentially the same restriction patterns as pFDGGT11 and varied only in the length at the termini. The clone with the longer cDNA insert, named pFDGGT53, was further studied. As shown in Fig. 2, about 1,500 and 500 bp of sequences at the 5' and 3' ends, respectively, were determined, which were completely identical with those of pFDGGT11 in the comparable regions. A sequence of 832 bp was extended from the 5' end of pFDGGT11, where the NH₂-terminus sequence of the Fd-GOGAT protein was found. At the 3' region, the position of the poly(A) tail addition was located 22 bp downstream of the terminal sequence of Fd-GOGAT. The combined sequence of pFDGGT11 and pFDGGT53 revealed the sequence of 5,617 bp long, and contained an open reading frame beginning at an ATG codon at nucleotide 328 encoding 1,616 amino acids. The mature protein starts from Cys98 and consists of 1,519 amino acids with a molecular weight of 165,298 in good agreement with the value calculated from the mobility of the polypeptide on SDS-polyacrylamide gel electrophoresis. Fd-GOGAT has been shown to be localized in chloroplasts and a leader sequence, presumably involved in the import of this protein into the organelle, is likely to be present at the NH₂ terminus. The sequence upstream of the codon for Cys98 seems to code the signal peptide. The ATG codon at nucleotide 328 presumably codes for the initiator methionine, since there is no other ATG codons in the upstream region. Thus, the cDNA clone pFDGGT53 seems to code for the entire precursor protein.

Genomic Organization of Fd-GOGAT Gene—Total genomic DNA was digested with five restriction enzymes, BamHI, EcoRI, KpnI, PstI, and XbaI, and resulting DNA fragments hybridized separately with two DNA fragments, probe I (670 bp) and probe II (1140 bp) which were located near the 5' and 3' ends of the coding sequence of pFDGGT11, respectively (see Fig. 2). As shown in Fig. 4, all digest, except PstI digest, showed single hybridization bands; two Pst fragments hybridized with probe II. These results were unchanged under lower stringency conditions of hybridization. The sizes of restriction fragments detected with the two probes were different from each other, suggesting that Fd-GOGAT gene was split by the digestions. Of the restriction enzymes used, there were no EcoRI or KpnI sites between the two DNA inserts used as probes in the sequence of pFDGGT11, and no PstI sites within probe II. These results indicate that Fd-GOGAT is most probably coded by a single gene and that at least two intervening sequences must be present within its coding region.

Northern Blot Analysis—The size and abundance of the Fd-GOGAT transcript in etiolated and greening leaves were determined by Northern blot analysis using probe I. Poly(A)⁺ RNA was prepared from the leaves of young seedlings grown for 6 days in the dark or subsequently greened for an additional 4 days. As shown in Fig. 5, a single major band around 5.3-5.5 kb in length was detected, and the signal intensity in greening leaves was about eight times that of etiolated leaves, indicating the accumulation of Fd-GOGAT mRNA was increased considerably by illumination. The size of the hybridizing band was in good agreement with that of the longest cloned cDNA, pFDGGT53. Again, this result fits in with the previous conclusion that the clone contains essentially the full-length cDNA.

**DISCUSSION**

In this paper we report, for the first time, the cloning and characterization of a cDNA encoding Fd-GOGAT. The identity of the cDNA is established by the coincidence of the structure of the purified protein with that inferred from the nucleotide sequence of the cDNA. The cDNA potentially encodes a polypeptide of 1616 amino acids and the deduced sequence contains two stretches of amino acids determined by NH₂-terminal sequencing of the Fd-GOGAT and its limited-cleavage fragments. The size and amino acid composition of the deduced polypeptide are very close to the values determined by analysis of the authentic protein.

Codon bias is found in some genes of monocots and they almost exclusively use codons ending in G and C (Campbell and Gowri, 1990). Genes for maize nitrate reductase (Gowri and Campbell, 1989) and nitrite reductase (Lahners et al., 1988), for example, show strong bias toward XXG/C codons (98 and 87%, respectively). The Fd-GOGAT gene from maize uses all sets of codons for the 20 amino acids and no such bias is observed (36% XXG/C codons).

Fd-GOGAT is a nuclear-encoded protein localized in the chloroplast stroma. Therefore, one would expect the polypeptide of Fd-GOGAT should be synthesized as a larger precursor, although no definite difference between the molecular size of the mature protein and an in vitro-synthesized precursor peptide was reported (Commere et al., 1986). The NH₂-terminal sequence analysis of Fd-GOGAT showed that the cysteine at amino acid position 98 is the NH₂-terminus of the
mature protein, and a potential initiation codon upstream of the cysteine residues is found (Fig. 3). Therefore, the mature Fd-GOGAT must be preceded by a transit peptide composed of 97 amino acids. Transit peptides of various precursor proteins share several features despite the lack of sequence similarities (Keegstra and Olsen, 1988). They are rich in the hydroxylated amino acids and small hydrophobic amino acids such as alanine and valine, and have a net positive charge. All these features are found in the sequence of the 97 amino acids, and the transit peptide of Fd-GOGAT, which is one of the longest proteins so far known to be imported into chloroplasts, seems to have the common characteristics.

A computer search revealed one significant sequence similarity between the maize Fd-GOGAT and the large subunit of the E. coli GOGAT (Oliver et al., 1987). As shown in Fig. 6, the best alignment of the two sequences gives an overall sequence homology; 633 residues out of a total of 1519 amino acids in the mature polypeptide of the maize enzyme are identical with those of the E. coli enzyme. In the E. coli enzyme, the sequence of 42 amino acid residues is removed to generate cysteine residue at the NH2-terminus of the active enzyme (Oliver et al., 1987). There is no significant sequence similarity between the transit peptide of Fd-GOGAT and the sequence of E. coli GOGAT, indicating that the processing of the transit peptide of the maize enzyme involved in chloroplast targeting is not directly related to the maturation of the E. coli enzyme. The E. coli GOGAT is an iron-sulfur flavoprotein containing FAD and FMN, and utilizes NADPH as a reductant (Miller and Stadtman, 1972). Fd-GOGAT from higher plants also contains iron-sulfur, FAD, and FMN as prosthetic groups, but reduced ferredoxin is used as an electron donor instead of pyridine nucleotides (Hirasawa and Tamura, 1984; Marquez et al., 1988). The sequence similarity extending over the entire region, and the same combination of the prosthetic groups, indicate that Fd-GOGAT and E. coli GOGAT may have a common intramolecular electron transfer pathway in the catalytic reaction. Fd-GOGAT is a monomeric protein and does not contain a smaller subunit similar to the E. coli enzyme. One would speculate that the difference in molecular configuration of the two enzymes might be related to the different specificity for the electron donors.

In addition, the homology search also found a significant match in a region from Leu176 to Arg2125 of Fd-GOGAT and

--Fig. 6. Comparison of amino acid sequences of maize Fd-GOGAT and the large subunit of E. coli NADPH-GOGAT. The amino acid sequence of the precursor protein of maize Fd-GOGAT (the upper sequence) is compared with that of the large subunit of E. coli NADPH-GOGAT (the lower sequence) (Oliver et al., 1987). The amino acid residues of the latter protein are shown only as dashes are introduced to give maximum homology. An arrow indicates the processing sites of the precursors.
that from Leu⁵⁶ to Arg⁴⁴ of yeast flavocytochrome b₁ (Lederer, 1985) as shown in Fig. 7. Flavocytochrome b₁ carries one molecule of FMN, and the above region is shown to be one of the candidates for a portion of the FMN-binding domain (Lederer, 1985), where a glycine-rich sequence, necessary for the formation of phosphate-binding loops, is contained (Walker et al., 1982). These characteristics are observed in the corresponding regions of both GOGAT enzymes. We tentatively assign the stretch of 58 amino acids located at the COOH-terminal half of the GOGAT proteins to be involved in FMN binding. No obvious homology was found between Fd-GOGAT and FAD-containing proteins or iron-sulfur proteins. In most cases, cysteine residues are involved in the binding to the iron-sulfur clusters of various types of Fd (Adaman et al., 1973; Fukuyama et al., 1980; Fukuyama et al., 1988). If this is also the case for the cluster formation of GOGAT, it is noteworthy that 9 cysteine residues at positions 98, 268, 688, 829, 1229, 1235, 1240, 1456, and 1494 corresponding to the numbering of the maize Fd-GOGAT are conserved between the maize Fd-GOGAT and the E. coli NADH-GOGAT.

NADH-GOGAT from higher plants is known to be an iron-sulfur flavoprotein with a single subunit of molecular mass of around 220 kDa (Suzuki and Gadal, 1984). The antibodies raised against maize Fd-GOGAT failed to cross-react with the maize NADH-GOGAT, suggesting that the two enzymes are structurally distinct proteins (data not shown). It will be interesting to know how the specificity for the electron donors is determined in the Fd- and pyridine nucleotide-dependent enzymes. At least maize Fd-GOGAT and E. coli NADPH-GOGAT are so similar that critical differences related to the specificity between the two enzymes cannot be predicted at present on a structural basis alone. Elucidation of the structure of the NADH-linked enzyme may give a clue to understanding the functional and evolutionary relationships between the two types of GOGAT from higher plants and bacteria.

The cloning of the cDNA for Fd-GOGAT led to an analysis of its mRNA levels in plants under different physiological conditions. The data presented here show that the amount of Fd-GOGAT message is about 8-fold higher in greening leaves compared with etiolated leaves (Fig. 5). A time course experiment indicated that this increase starts within 6 h after illumination of etiolated seedlings and that the accumulation of the Fd-GOGAT polypeptide is preceded by mRNA accumulation. These results suggest that a transcriptional control is involved in the light induction of the enzyme activity. This phenomenon is consistent with many previous observations that both enzyme activity and protein levels increase considerably with greening (Suzuki et al., 1982; Wallsgrove et al., 1982; Suzuki et al., 1987).

Fd-GOGAT has also been detected in non-green tissues and the enzyme from rice roots exhibits immunological and kinetic properties different from the leaf enzyme, suggesting the presence of isoforms (Suzuki et al., 1982). Fd, the electron donor to the enzyme, is also shown to exist as a multiform in maize, and the compositions of the isoproteins differ between leaves and roots (Kimata and Hase, 1989). Our genomic hybridization results, however, gave no indication that more than one Fd-GOGAT gene is present in maize genome. Northern hybridization of poly(A)⁺ RNA from leaves and roots gave the same size of signal for the Fd-GOGAT transcript.

Further experiments are now in progress to obtain a genomic clone for Fd-GOGAT and to study mechanisms of gene expression and response to light for the accumulation of the transcript in photosynthetic and non-photosynthetic tissues.

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SUPPLEMENT TO MOLAR MATERIALS AND METHODS

Plant Material: Winter maize (Zea mays L. ssp. mays B. Hort) seedlings were grown on commission at 25°C in a dark room for 4 days. The 2-month seedlings were then transferred to a growth chamber and continuously illuminated with fluorescent lights (6.5 W per m²) until harvest. At harvest, the mature leaves were peeled from the stem and immediately frozen in liquid nitrogen. After thawing, the leaves were ground to a fine powder in liquid nitrogen and stored at −20°C until use.

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Plant Material... Winter maize (Zea mays L. ssp. mays B. Hort) seedlings were grown on commission at 25°C in a dark room for 4 days. The 2-month seedlings were then transferred to a growth chamber and continuously illuminated with fluorescent lights (6.5 W per m²) until harvest. At harvest, the mature leaves were peeled from the stem and immediately frozen in liquid nitrogen. After thawing, the leaves were ground to a fine powder in liquid nitrogen and stored at −20°C until use.
RESULTS

The NH2-terminal sequences and amino acid compositions of Fd-GOGAT. 88 kDa fragment, and 73 kDa fragment were shown in Table S.1. Amino acid sequences deduced from the NH2-terminal sequence was found for Fd-GOGAT and 73 kDa fragment and this corresponded to a region from Gly 5 to Met 494 in the amino acid sequence deduced from the nucleic acid sequence of the cloned cDNA (Fig. 2) in text). The NH2-terminal sequence of the 88 kDa fragment was found in the region starting from Gly5 of the deduced sequence. Although the first and 9th residues of Fd-GOGAT and the 73 kDa fragment were not the same due to N-terminal deamination and lysine, these residues were not identified, the sequencing results are reasonably explained, because these residues generally show a loss recovery of their phenylthiohydantoin derivatives during Edman degradation. Differences of the amino acid compositions of the 88 kDa and the 73 kDa fragments were apparent, especially in values of glycine, alanine, and isoleucine, and the sum of each residue was in good agreement with the composition obtained from the original fragment. The 73 kDa and 88 kDa fragments are originated from a different region of Fd-GOGAT.

In order to examine whether the 88 kDa fragment and the 73 kDa fragment were antigenically different, antibodies raised against Fd-GOGAT were affinity-purified with each of the fragments and reactivities of the epitope-selected antibodies were determined. The results are shown in Fig. S-1. The antibodies purified with Fd-GOGAT strongly recognized the two fragments, as well as Fd-GOGAT, whereas those selected with the fragment showed only a trace of the reactivity against the counterpart of each fragment in comparison with the reactivities towards the original fragments. Therefore, the two fragments were antigenically different from each other.

![Figure S-1. Detection of Fd-GOGAT, the 88 kDa fragment, and the 73 kDa fragment with epitope-selected antibodies. The semi-purified protein from Fd-GOGAT was separated by SDS-PAGE as shown in lane 1. The 88 kDa fragment and the 73 kDa fragment were transferred to Immobilon-P and blocked using 10% skim milk. The fragments were detected using Coomassie brilliant blue (lane 1) and Western blotted using epitope-selected antibodies with Fd-GOGAT (lane 4), the 88 kDa fragment (lane 2), or the 73 kDa fragment (lane 3).]

Table S-1

| Cycle | Fd-GOGAT | 88 kDa fragment | 73 kDa fragment |
|-------|----------|-----------------|-----------------|
| 1     | n. d.    | Gly (249)       | n. d.           |
| 2     | Gly (96) | Lys (40)        | Gly (249)       |
| 3     | Val (83) | Met (65)        | Val (123)       |
| 4     | Gly (97) | Pro (67)        | Gly (165)       |
| 5     | Phe (62) | Thr (26)        | Phe (110)       |
| 6     | Val (98) | Val (80)        | Val (145)       |
| 7     | Ala (82) | Thr (47)        | Ala (186)       |
| 8     | Asx (32) | Ile (94)        | Asx (90)        |
| 9     | Leu (136)| Glu (138)       | Leu (207)       |
| 10    | n. d.    | Glu (76)        | n. d.           |
| 11    | Asx (50) | Ala (111)       | Asx (68)        |
| 12    | Met (26) | Glu (62)        | Met (196)       |

Approximately 500 pmol of each polypeptide was subjected to sequence analysis. Yields are shown in parentheses in pmol.

Table S-11

| Amino acid 88 kDa fragment | 73 kDa fragment | Fd-GOGAT | pFDGGT53 |
|---------------------------|-----------------|----------|----------|
| Asx                       | 76              | 147      | 141      |
| Thr                       | 43              | 83       | 73       |
| Ser                       | 49              | 51       | 104      | 105     |
| Glu                       | 99              | 82       | 176      | 159     |
| Pro                       | 35              | 29       | 64       | 72      |
| Gly                       | 109             | 61       | 157      | 142     |
| Ala                       | 73              | 53       | 122      | 130     |
| Val                       | 59              | 42       | 100      | 115     |
| Met                       | 7               | 10       | 15       | 37      |
| Ile                       | 61              | 38       | 94       | 85      |
| Leu                       | 82              | 78       | 154      | 145     |
| Tyr                       | 13              | 10       | 29       | 41      |
| Phe                       | 26              | 26       | 52       | 50      |
| Lys                       | 46              | 36       | 75       | 77      |
| His                       | 13              | 9        | 22       | 26      |
| Arg                       | 37              | 37       | 67       | 78      |
| Trp                       | n. d.           | n. d.    | n. d.    | 17      |
| Cys                       | n. d.           | n. d.    | n. d.    | 26      |

Total: 1519

Calculations are based on the apparent molecular masses of each polypeptide.

*pAmino acid composition of the mature Fd-GOGAT deduced from cDNA clone, pFDGGT53*.

*nNot determined*