Short Communication

Molecular Cloning and Partial Amino Acid Sequence of Rice Ferredoxin-Nitrite Reductase

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The rice plant is known to use ammonium in preference to nitrate as a nitrogen source.1 However, nitrate is also assimilated when it is supplied as a single nitrogen form.2 In nitrate nutrition, it must be reduced to ammonium through two enzymatic reactions. NR is the first enzyme catalyzing the reduction of nitrate to nitrite, followed by nitrite reduction to ammonium by Fd-NiR.

The molecular and enzymatic nature of these two enzymes has been extensively characterized and recently much attention has been directed to the analysis and regulation of the nitrate-nitrite assimilatory genes from higher plants and fungi.3 However, there has been only limited work on the enzymatic and genetic aspects of these enzymes in rice. Recently the genomic DNA sequence of rice NR has been reported.4 We described previously the in vitro synthesis and induction of rice NiR by nitrate, indicating that the induction of the enzyme’s activity and protein was brought about at the transcriptional and/or translational level of the NiR gene.5 As a first step in elucidating the gene structure and its regulation of the nitrate-assimilating system in rice, we have attempted molecular cloning of rice Fd-NiR and report here comparisons of partial cDNA and amino acid sequences of the enzyme with those known for NiR from maize6 and spinach.7

The isolation and characterization of poly(A)⁺ RNA from nitrate-treated greening rice seedlings have been described in detail elsewhere.5 Double-stranded cDNA was synthesized from the mRNA using a cDNA kit (Amersham International) as described.8 A cDNA library was constructed in λgt11 using a ligation kit (Takara Shuzo) and a packaging kit (Gigapack, Stratagene) as described.9 The rice leaf cDNA library was immunoscreened with anti-rice NiR antiserum prepared previously10 using alkaline phosphatase-conjugated rabbit antibodies. Antibody-positive phage clones were further screened by hybridization to a maize NiR cDNA clone (1.9 kb, provided by Dr. S. Rothstein, University of Guelph, Canada) as a DNA probe. The maize NiR cDNA was labeled with [³²P]-dCTP using a random primer labeling kit (Takara Shuzo). Southern hybridization was done for high-abundance sequences as described.11 EcoR1 restriction fragments from recombinant phage that reacted with both antibodies and DNA probe were sized in 1% agarose, isolated by electroelution and subcloned into pUC 119 as described.11 DNA was sequenced with the denatured DNA as a template12 by the deoxy chain-termination technique.13 For amino acid sequencing, homogeneous rice Fd-NiR10 was digested with endoproteinase Lys-C (Boehringer Mannheim) as described.14 Peptide mixtures from the enzymatic digestion were separated by reverse-phase HPLC with a Shimadzu LC-4A apparatus using a Spherisorb ODS column (Merck, Darmstadt) and trifluoroacetic/acetonitrile system. The amino acid sequence was analyzed with an Applied Biosystem model 470 sequencer for peptides that were shown to be single peaks on chromatograms. The C-terminal sequence was identified as described.15

Fifty antibody-positive cDNA clones were

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Abbreviations: Fd, ferredoxin; NiR, nitrite reductase; NR, nitrate reductase.
isolated from a portion (1.8 × 10^5 plaques) from the leaf cDNA library. Ten clones were randomly chosen for further cloning with the maize NiR cDNA, resulting in 8 recombinant phage clones. The DNA sequence for one of these clones carrying 0.5 kb showed that the 5' end 288 nucleotide sequence was the coding region matched with the C-terminal sequence (-Asp-Glu-Glu) known for the enzyme protein, while 3' end 202 bases were the untranslated tail region starting at the TAG termination codon including a putative polyadenylation signal, but no poly(A) tail (Fig. 1). In comparison of the same coding sequences, the rice clone has a 89% homology to the maize NiR cDNA, while the content of G/C in the spinach cDNA has been reported to be 44%. Unique codon usage has been well documented for the monocot genes. On the basis of the protein sequence of the same coding segments, the deduced amino acid sequence of the rice NiR cDNA show a 90% homology to the maize enzyme. The degree of protein sequence conservation decreases to 70% identical amino acid to spinach NiR. A high degree of homology of the primary structure is also found in a peptide fragment (50 amino acid residues) from the rice enzyme, corresponding to positions 392 to 441 of the spinach enzyme, where there were 95% and 88% identical sequences to the maize and spinach enzymes, respectively (Fig. 2).

Although the protein sequences deduced from this clone and from a peptide fragment of the enzyme carry only limited segments of the entire sequence, high degrees of sequence
Fig. 2. Comparison of Amino Acid Sequences of Fd-NiR from Rice (1), Maize (2), and Spinach (3). Positions 392 to 441 of the rice enzyme were sequenced for the peptide fragment. Numbers below the spinach enzyme are given to the amino acid residues predicted from its cDNA.7) The sequence for the maize enzyme is from ref. 6.

Homology can be expected throughout the whole primary structure of NiR from rice, maize, and spinach, as it has been indicated between the latter two proteins6) and suggested in amino acid compositions.10) It has been mentioned that considerable variations have occurred at the N-terminal region of the mature proteins.6) The molecular weight of rice NiR is lower by 2000 than that of the spinach enzyme.10) It is interesting to find out the difference in molecular size in the primary structure. Analysis of a full-length NiR cDNA may be instrumental in elucidating those unclear aspects of rice Fd-NiR.

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References