

Plant Gene Register

Nucleotide Sequences of cDNA Clones Encoding Ferrochelatase from Barley and Cucumber¹Kazumasa Miyamoto, Ryoichi Tanaka, Haruhiko Teramoto, Tatsuru Masuda², Hideo Tsuji, and Hachiro Inokuchi*

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Ferrochelatase is the enzyme that catalyzes the last step in the heme biosynthetic pathway. It catalyzes the insertion of iron (Fe²⁺) into the tetrapyrrole ring of protoporphyrin IX to generate protoheme. Recently, we isolated mutants of *Escherichia coli* that were sensitive to visible light (Miyamoto et al., 1991; Nakahigashi et al., 1991), and these mutants [designated *visA* (= *hemH*) mutants] were shown to be the result of a mutation in the *visA* (= *hemH*) gene, which encodes ferrochelatase. The nucleotide sequences of genes that encode ferrochelatases from six different sources are available (*E. coli*, Miyamoto et al., 1991; *Bacillus subtilis*, Hansson and Hederstedt, 1992; *Bradyrhizobium japonicum*, Frustaci and O'Brian, 1992; yeast, Labbe-Bois, 1990; mouse, Taketani et al., 1990; human, Nakahashi et al., 1990), but none have been reported from higher plants.

Here we report the isolation and the sequences of cDNAs that encode a ferrochelatase (protoheme ferro-lyase, EC 4.99.1.1) from barley (*Hordeum vulgare* L. cv Svalöf's Bonus) and a ferrochelatase from cucumber (*Cucumis sativus* L. cv Aonagajibai). Both cDNAs were isolated from libraries in phage vector λ gt11. To screen the clones, we used a deletion mutant of *E. coli* (strain VS200), in which part of the gene for ferrochelatase had been deleted. Because VS200 mutants produce no heme, they cannot grow well even on complete medium. Therefore, we isolated large colonies of VS200 cells that formed among the tiny colonies after infection by the clones in the cDNA libraries of barley and cucumber. Phages obtained from the large colonies were tested for their ability to improve the poor growth of VS200 bacteria. To determine the nucleotide sequence of the cDNA insert in each selected phage clone, an *EcoRI* fragment was recloned into plasmid vector pUC118 and serial-deletion clones were constructed by the step-wise method of Yanisch-Perron et al. (1985).

The insert of a phage clone from the barley cDNA library was 1728 bp long and included a single open reading frame that encoded a polypeptide of 484 amino acids (Table I). Coincidentally, the insert from the cucumber cDNA library

Table I. Characteristics of cDNAs that encode ferrochelatases from barley and cucumber

Organism:	Barley; <i>Hordeum vulgare</i> L. cv Svalöf's Bonus.					
	Cucumber; <i>Cucumis sativus</i> L. cv Aonagajibai.					
Enzyme, Function:	Ferrochelatase (protoheme ferro-lyase; EC 4.99.1.1); insertion of a ferrous ion into protoporphyrin IX.					
Source of Clones:	cDNA library in λ gt11.					
Techniques:	Screening of cDNA clones: by complementation with a deletion mutant of <i>E. coli</i> (strain VS200), in which part of the gene for ferrochelatase is missing.					
	Nucleotide sequencing: after construction of serial-deletion clones (using pUC118) by the step-wise method, both strands were sequenced by the dideoxy chain-termination method.					
Features of cDNA Structures:	Barley: contains 1728 nucleotides: a 90-nucleotide 5' untranslated region; a 1452-nucleotide open reading frame; and a 186-nucleotide 3' untranslated region.					
	Cucumber: contains 1763 nucleotides: a 94-nucleotide 5' untranslated region; a 1542-nucleotide open reading frame; and a 127-nucleotide 3' untranslated region.					
Features of Predicted Amino Acid Sequence:	Barley: open reading frame of 484 amino acids; predicted molecular mass 53,472 D (including the leader peptide).					
	Cucumber: open reading frame of 514 amino acids; predicted molecular mass 57,206 D (including the leader peptide)					
Amino Acid Sequence Similarity (%):						
	<i>E. coli</i>	<i>B. subtilis</i>	<i>B. japonicum</i>	Yeast	Mouse	Human
Barley	32.0	30.5	29.7	36.1	39.6	38.4
Cucumber	31.4	30.5	29.1	35.3	40.1	40.1

was almost the same size, 1763 bp, and it contained a single open reading frame (514 amino acids; Table I). From a homology search with the amino acid sequences of ferrochelatases from eukaryotes (yeast, mouse, and human), we predicted the amino terminus of the mature ferrochelatases. The similarity of the ferrochelatases from barley and cucumber is about 77% at the amino acid level. The similarity between these two ferrochelatases and those from other sources is also indicated (Table I). The predicted molecular masses of the mature ferrochelatases of barley and cucumber are approximately 43 and 46 kD, respectively.

¹ This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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Received January 4, 1994; accepted January 31, 1994.

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The DDBJ/EMBL/GenBank accession numbers of the sequences reported in this article are D26105 (barley) and D26106 (cucumber).

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