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Soybean Nodule-Specific Uricase (Nodulin-35) Is Expressed and Assembled into a Functional Tetrameric Holoenzyme in *Escherichia coli*[1]

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ABSTRACT

A complete nodulin-35 (N-35) cDNA encoding nodule-specific uricase (EC 1.7.3.3) was isolated from a soybean (*Glycine max* L. var. Prize) nodule cDNA expression library using a previously isolated partial cDNA clone. The N-35 cDNA was expressed in *Escherichia coli* driven by the *lacZ* promoter and was found to be functionally active. The uricase activity was detected in the cytoplasmic fraction of *E. coli* with the same pH optimum and apparent K_m values as that in the nodules. Because a stop codon is located 15 base pairs upstream of the N-35 initiation codon, it appears that a fusion protein with LacZ was not made, but reinitiation occurred due to the presence of a putative Shine-Dalgarno sequence in the appropriate region. The size of the N-35 polypeptide made in *E. coli* is identical to that present in soybean nodules and is able to assemble into a tetrameric holoenzyme with the same molecular weight as the native uricase. Thus, the presence of peroxisomes does not appear to be essential for the proper assembly of the holoenzyme in *E. coli.* These data also indicate that posttranslational modifications or membrane transport are not essential either for the assembly of N-35 into a holoenzyme or for the activity of uricase.

Tropical legumes, such as soybean, assimilate symbiotically fixed nitrogen into ureides that are translocated to the shoot (17). Uricase (EC 1.7.3.3) is a key enzyme involved in ureide production, and the nodule form of this enzyme (uricase-II) is different from that present in uninfected young seedlings (1, 21). Uricase catalyzes the conversion of uric acid to allantoin, a reaction carried out in the peroxisomes of uninfected cells of nodules (15, 16). N-35[2] is a subunit of uricase-II and is a major protein in soybean root nodules. It is primarily localized in the peroxisomes of uninfected cells of the infection zone and the inner cortex (our unpublished observations). Because the ureide biosynthetic pathway is compartmentalized between infected and uninfected cells (17, 23) it appears that an intermediate metabolite of this pathway is involved in the induction of the N-35 gene.

The nodule-specific uricase has its pH optimum at 9.5 and the native enzyme contains four similar subunits having ~1.0 mol Cu^{2+} per subunit (1). N-35 is translated on free polysomes in the host cell cytoplasm, and posttranslationally translocated into the peroxisome without any apparent modification such as processing and glycosylation (16). The posttranslational transport has also been observed for other peroxisomal proteins (10). Using a genetic approach, Gould *et al.* (6) demonstrated that one animal peroxisomal enzyme, firefly luciferase (EC 1.13.12.7), contains the peroxisomal targeting signal at its carboxyl terminus. A homologous amino acid sequence has been observed in the carboxyterminus of several peroxisomal proteins, and Miyazawa *et al.* (14) proposed the importance of the three amino acid sequence Ser-Lys-Leu-COOH at the carboxyl terminus of these proteins for translocation to the peroxisomes.

We are interested in the assembly and targeting mechanism of N-35 to the peroxisomes and in the manner in which these organelles are induced in the uninfected cells of nodules. We have earlier isolated a cDNA clone of N-35 and deduced its primary structure (16). Here we demonstrate that a complete N-35 cDNA, when expressed in *Escherichia coli,* produces a functional enzyme that remains soluble in the cytoplasmic fraction. Furthermore, we suggest from these results that targeting to peroxisomes is not essential for the correct assembly of N-35 into a tetrameric holoenzyme and subsequent enzymatic activity.

MATERIALS AND METHODS

Growth of Plants and Bacteria

Soybean (*Glycine max* L. var. Prize) seeds were inoculated with *Bradyrhizobium japonicum* (61A76) and grown as previously described (21). Nodules were harvested 3 weeks after inoculation and stored in liquid nitrogen until used.

Escherichia coli (JM101) carrying a complete N-35 cDNA (pHN35) driven by *lacZ* promoter was grown overnight at 37°C in 100 mL of 1.5% Bacto-tryptone, 0.5% NaCl including 50 μg/mL of ampicillin. This culture was inoculated into 900 mL of the same medium containing 1 mM isopropyl-l-thio-β-D-glactoside and incubated for 5 h with shaking at 37°C. Bacterial cells were harvested by centrifugation at 5000*g* and stored at −80°C until used.

cDNA Cloning

Poly(A)$^+$ RNA was purified from 3-week-old soybean nodules. Double-stranded cDNAs were cloned into the *Xho*I site of λZapII (Stratagene, CA) as described earlier (4). An 800-

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[2] Abbreviations: N-35, nodulin-35; kb, kilobase pair.

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Dare:          TTCAGTTCTAAGAAACCTTAAAAA ACAA GT   AGTGTTCCGAAAAG
Prize:         -C-G-----C--------------CA----C--AGG-------G------

       M   A   Q   Q   E   V   V   E   G   F   K   F   E   Q   R
Dare:  ATG GCT CAG CAG GAA GTG GTA GAA GGG TTC AAG TTC GAA CAG AGG
Prize: --- --- --- --- --- --- --G --- --- --- --- --- --G --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   .45

       H   G   K   E   R   V   R   V   A   R   V   W   K   T   R
Dare:  CAC GGG AAA GAG CGC GTG AGA GTG GCG CGC GTG TGG AAG ACG AGG
Prize: --- --- --- --A --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   .90

       Q   G   Q   H   F   V   V   E   W   R   V   G   I   T   L
Dare:  CAG GGG CAG CAC TTC GTT GTG GAG TGG CGC GTG GGC ATC ACT CTC
Prize: --- --- --- --- --- A-- --- --- --- --- --- --G --- --- ---
       .   .   .   .   .   I   .   .   .   .   .   .   .   .   135

       F   S   D   C   V   N   S   Y   L   R   D   D   N   S   D
Dare:  TTC TCA GAC TGC GTC AAC TCG TAT CTC CGC GAT GAC AAC TCT GAC
Prize: --T --G --T --- --- --- --- --C --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   180

       I   V   A   T   D   T   M   K   N   T   V   Y   A   K   A
Dare:  ATC GTT GCT ACT GAT ACC ATG AAA AAC ACA GTG TAT GCA AAA GCA
Prize: --- --- --- --- --- --- --- --- --- --C --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   225

       K   E   C   S   D   I   L   S   A   E   D   F   A   I   L
Dare:  AAG GAA TGC TCA GAC ATA CTC TCT GCT GAG GAC TTT GCT ATT TTG
Prize: --- --- --- --T --- --- --T --- --C --- --G --- --- --- C--
       .   .   .   .   .   .   .   .   .   .   E   .   .   .   270

       L   A   K   H   F   V   S   F   Y   K   K   V   T   A   A
Dare:  CTT GCT AAG CAC TTT GTA TCA TTT TAC AAG AAG GTT ACT GGT GCT
Prize: --- --- --- --- --- --- --- --- --- C-- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   Q   .   .   .   .   315

       I   V   N   I   V   E   K   P   W   E   R   V   I   V   D
Dare:  ATT GTG AAT ATT GTG GAA AAA CCA TGG GAG CGT GTC ATT GTG GAT
Prize: --- --- --- --- --- --- --- --- --- --- --- --- -C- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   T   .   360

       G   Q   P   H   E   H   G   F   K   L   G   S   E   K   H
Dare:  GGT CAA CCT CAT GAA CAT GGT TTC AAA CTT GGG TCT GAG AAG CAT
Prize: --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   405

       T   T   E   A   I   V   Q   K   S   G   S   L   Q   L   T
Dare:  ACA ACA GAG GCA ATA GTA CAA AAG TCT GGT TCA CTT CAG TTG ACT
Prize: --- --- --- --G --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   450

       S   G   I   E   G   L   S   V   L   K   T   T   Q   S   G
Dare:  TCT GGT ATT GAA GGA TTG TCA GTG TTG AAG ACA ACC CAG TCT GGT
Prize: --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   495

       F   V   N   F   I   R   D   K   Y   T   A   L   P   D   T
Dare:  TTT GTG AAT TTC ATA AGA GAC AAG TAC ACA GCA CTT CCT GAT ACC
Prize: --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   540

       R   E   R   I   L   A   T   E   V   T   A   L   W   R   Y
Dare:  CGT GAA AGG ATT CTG GCA ACA GAA GTA ACC GCA CTG TGG AGG TAT
Prize: --- --- --- --G G-A --- --- --- --- --- --- --- --- --- ---
       .   .   .   M   V   .   .   .   .   .   .   .   .   .   585

       S   Y   E   S   Q   Y   S   L   P   Q   K   P   F   Y   F
Dare:  TCG TAT GAA TCA CAG TAT AGC CTC CCT CAG AAG CCA TTT TAC TTT
Prize: --- --- --- --G -T- --- --- --- --- --- --- --G C-- --- ---
       .   .   .   .   L   .   .   .   .   .   .   .   L   .   630

       T   E   K   Y   Q   E   V   K   K   V   L   A   D   T   F
Dare:  ACA GAA AAG TAT CAG GAA GTG AAA AAA GTT CTG GCT GAC ACT TTT
Prize: --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   675

       F   G   P   P   N   G   G   V   Y   S   P   S   V   Q   N
Dare:  TTT GGC CCA CCA AAT GGG GGA GTC TAT AGC CCA TCT GTT CAA AAC
Prize: --- --- --- --- --A --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   K   .   .   .   .   .   .   .   .   .   720

       T   L   Y   L   M   A   K   A   T   L   N   R   F   P   D
Dare:  ACA CTC TAC CTG ATG GCA AAG GCC ACA CTG AAC AGA TTT CCT GAC
Prize: --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   765

       I   A   Y   V   S   L   K   M   P   N   L   H   F   L   P
Dare:  ATA GCT TAT GTC AGT CTA AAG ATG CCA AAT CTT CAT TTC TTA CCT
Prize: --- --- --- --- --- --- --- T-- --- --- --- --- --- A-- ---
       .   .   .   .   .   .   .   L   .   .   .   .   .   I   810

       V   N   I   S   N   Q   D   G   P   I   V   K   F   E   D
Dare:  GTC AAT ATC TCA AAC CAG GAT GGT CCT ATT GTG AAG TTT GAG GAT
Prize: --- --- --- --- --- --- --- --C --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   855

       D   V   Y   L   P   T   D   E   P   H   G   S   I   Q   A
Dare:  GAT GTG TAC TTG CCA ACG GAT GAG CCT CAT GGG TCA ATT CAA GCT
Prize: --- --- --- --- --- --- --- --- --A --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   .   .   .   .   .   900

       S   L   S   R   L   W   S   K   L
Dare:  AGC TTG AGC CGC CTT TGG TCA AAG CTG TAG
Prize: --T --- --- --- --- --- --- --- --- ---
       .   .   .   .   .   .   .   .   .   930
```

Figure 1. Comparison of nodulin-35 sequence from two varieties of soybean. A complete nodulin-35 cDNA clone from soybean variety Prize was compared with the deduced cDNA of variety Dare (see ref. 16). A hyphen (-) indicates identical nucleotides and a period (.) indicates identical amino acids. Gaps are created in the 5′-end to align both sequences. The putative SD sequence is underlined by a thick line, and the TAG codon preceding the initiation codon is indicated by a thin line. The number refers to the coding sequence only.

base pair cDNA fragment coding for a part of N-35 (14) was prepared from pNOD35 and used as a probe for screening the λZapII library. The DNA probe was labeled with multiprime DNA labeling system (Amersham) using [α^{32}P]dCTP. Several positive plaques were obtained and λ phages were isolated. They were then converted into plasmids by rescuing (19), according to the procedure provided by Stratagene, Inc. (see also ref. 4 for details).

DNA Sequencing

A plasmid (pHN35) carrying about a 1.3-kb insert of N-35 cDNA was purified by CsCl ethidium bromide centrifugation and used for dideoxynucleotide chain termination sequencing. Denatured double strand plasmid DNA was sequenced using Sequenase Version II (U.S. Biochemicals, Cleveland, OH) and [α^{32}P]dATP. The sequence was compared with the deduced cDNA sequence from the variety Dare (16).

Purification of Uricase and Enzyme Assay

Soybean nodules were ground to a fine powder in liquid nitrogen. Ground tissue was resuspended in 1:4 (w/v) TSB (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 250 mM sucrose) and passed through two layers of Miracloth (Calbiochem). The filtrate was centrifuged for 30 min at 30,000*g* at 4°C to obtain the soluble protein fraction (S-30). Uricase activity from the S-30 fraction was precipitated with 30% saturation of ammonium sulfate (11) to obtain a P-30 fraction. The pellet was dissolved in a small amount of 0.1 M Tris-HCl (pH 8.7) and desalted on a G-50 column with 10 mM potassium phosphate buffer (pH 7.5). The desalted sample was loaded onto a DE52 anion exchange column (Whatman) equilibriated with 10 mM potassium phosphate buffer, pH 7.5. Under these conditions, N-35 passed through the DE52 column while most of the other proteins remained bound. The DEAE unbound fraction was reprecipitated with 30% saturation of ammonium sulfate and the pellet was dissolved in a small amount of 0.1 M Tris-HCl (pH 8.7) and desalted as above. Uricase activity expressed in *E. coli* carrying pHN35 was purified with the same procedure, following sonication of *E. coli* cells. Periplasmic proteins from *E. coli* were prepared as described by Koshland and Botstein (9).

The native size of uricase expressed in *E. coli* was determined by gel filtration chromatography. The DEAE unbound fraction was loaded onto a Superose-12 (Pharmacia) fast protein liquid chromatography column at a flow rate of 0.15 mL/min with 100 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl, or 10 mM potassium phosphate buffer (pH 7.5). The effluent was monitored at 280 nm followed by determination of uricase activity in each fraction. Gel filtration pro-

Table I. *Expression of Uricase Activity in E. coli Containing pHN35*

Bacteria	Fraction	Uricase Activity	
		Specific activity	Total activity
		nmol/min/μg protein	%
JM101	Total proteins	0.01	
JM101 (pHN35)	Total proteins	0.15	100.0
JM101 (pHN35)	Periplasmic proteins	0.04	2.0
JM101 (pHN35)	Cytoplasmic proteins	0.15	82.0

tein standards (Bio-Rad) were used to calibrate the column and to determine the native sizes of N-35 expressed in *E. coli* and the native uricase protein in nodules.

Uricase activity was measured as described previously (11). The assay mixture contained in 1 mL: 50 mM Tricine-KOH (pH 9.5), 0.1 mM uric acid (prepared fresh), and an appropriate amount of enzyme sample. The reaction was started with the addition of the enzyme sample, and uricase activity was detected spectrophotometrically following the reduction in absorbance of uric acid of 293 nm at 25°C. Protein concentrations were determined with a Bio-Rad protein assay reagent (2).

RESULTS AND DISCUSSION

Expression of Soybean Nodulin-35 cDNA in *E. coli*

A soybean (var. Prize) nodule cDNA expression library constructed in the λZapII vector was screened using a partial cDNA clone of N-35 isolated previously (16). More than 30 positive plaques were identified from 2×10^4 plaques. Three plaques showing a stronger signal than the other positive plaques were chosen. Those phages were isolated and converted to plasmids (pBluescript SK^+ derivatives) using a helper phage and a strategy called "rescue" (19) as described previously (4). These rescued plasmids carried about 1.25-to 1.3-kb DNA inserts. The coding region of N-35 (*Kpn*I-*Eco*RV fragment) was cut out from a clone in pBluescript and subcloned into *Kpn*I and *Sac*I sites of pUC19 after changing the *Eco*RV site to *Sac*I (using *Sac*I linker) to obtain a plasmid pHN35. The nucleotide sequence of a 1.3 kb-long cDNA was determined with the dideoxynucleotide chain termination method. In comparison with the sequence deduced from a genomic clone of N-35 from soybean variety Dare (16), the Prize N-35 cDNA contained a 50-nucleotide leader sequence flanking the coding region as shown in Figure 1. The two sequences showed 31 nucleotide differences in the coding region resulting in 11 amino acid differences. The 5′ leader of Prize cDNA showed 20% difference within 50 nucleotides. Because a termination codon (TAG) was located 15 nucleotides upstream of the N-35 initiation codon, this would not allow any fusion protein to be made. However, a purine-rich region which appears to function as a Shine-Dalgarno sequence (18) was observed four nucleotides upstream of the initiation codon of N-35, and it seems that reinitiation occurred due to the presence of this sequence making an unfused N-35 product initiated from proper methionine codon. This evidence is supported by the studies on the characterization of nodulin-35 protein (see below).

Subcellular Location of N-35 in the Absence of Peroxisomes

E. coli JM 101 carrying pHN35 was cultured in the presence of 1 mM isopropyl-l-thio-β-D-galactoside, and the uricase activity in the total cell extract was assayed. As shown in Table I, a high level of uricase activity was observed in the transformed bacteria. Wild-type *E. coli* does not have any uricase activity and shows a very low level of nonspecific activity. Total bacterial proteins from transformed and nontransformed cells were prepared using sonication and the same amount of proteins were analyzed on SDS-PAGE. A major protein band was detected in the transformed bacterial cell extract which comigrated with the N-35 from soybean root nodules (Fig. 2). This band is absent in untransformed *E. coli* (data not shown).

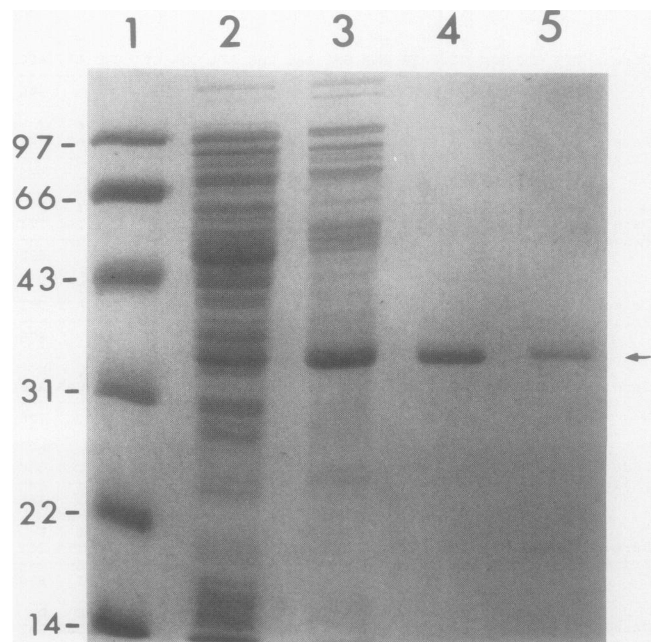

Figure 2. SDS-PAGE of proteins from *E. coli* at each purification step. Lane 1, mol wt standards; lane 2, 50 μg of S-30 from *E. coli* (pHN35); lane 3, 20 μg of P-30; lane 4, 5 μg of DEAE unbound fraction; lane 5, 3 μg of DEAE unbound fraction from soybean nodules. The gel was stained with Coomassie brilliant blue R after electrophoresis. The arrow indicates the N-35 protein band.

Table II. *Purification of N-35 from Nodules and E. coli Containing pHN35*

Origin	Purification Step	Protein		Uricase Activity	
				Specific activity	Recovery
		mg	%	*nmol/min/μg protein*	%
Nodule	S-30	126	100	0.17	100
	P-30	21	17	0.96	97
	DE52	1.2	0.95	4.5	32
E. coli (pHN35)	S-30	55	100	0.23	100
	P-30	3.3	5.9	1.5	40
	DE52	0.16	0.29	4.8	6.1

The subcellular location of N-35 in *E. coli* was determined by assaying soluble and periplasmic proteins as described by Koshland and Botstein (9). Table I shows that most of the activity was found to be located in the cytoplasmic fraction of the cells containing the pHN35 plasmid. While this protein is functional in *E. coli* cytoplasm, it is not active and did not accumulate in any significant amount when produced in tobacco cells under the control of CaMV-35S promoter (data not shown). In the absence of peroxisomes the N-35 in the cytoplasm may be inactive or degraded.

N-35 Expressed in *E. coli* is Structurally and Functionally Similar to That in Nodules

The uricase was purified from bacteria carrying pHN35 essentially as described earlier (1), and compared with the native soybean enzyme from nodules. The purification procedure is summarized in Table II and the purity of each fraction is shown in Figure 2. The results show that N-35 expressed in *E. coli* can be purified with the same procedure as used for the native soybean nodule enzyme. The DEAE unbound fraction of bacterial extract showed a major single protein band comigrating with the soybean N-35 protein. This data strongly suggested that the purine-rich region in front of the N-35 initiation codon functioned as a Shine-Dalgarno sequence forming an unfused N-35 protein which is enzymatically active in *E. coli*. However, a significant loss of N-35 from bacteria was observed at each step in comparison to that from root nodules (Table II). The differences in cellular condition, *e.g.* oxidation, and the differences in subcellular location of N-35 between the two organisms (cytoplasmic *versus* peroxisomal in *E. coli* and soybean, respectively) may be responsible for the differences in the recovery of this enzyme.

Several multi-subunit plant enzymes such as glutamine synthetase from *Medicago sativa* (3) and soybean (7), Δ^1-pyrroline-5-carboxylate reductase from soybean (5), and phytohemagglutinin from *Phaseolus vulgaris* (8) have been cloned and expressed functionally in *E. coli*. These proteins are correctly assembled in *E. coli* and form soluble holoenzymes with the same number of subunits as in plants. Because uricase is localized in a specialized subcellular compartment where it

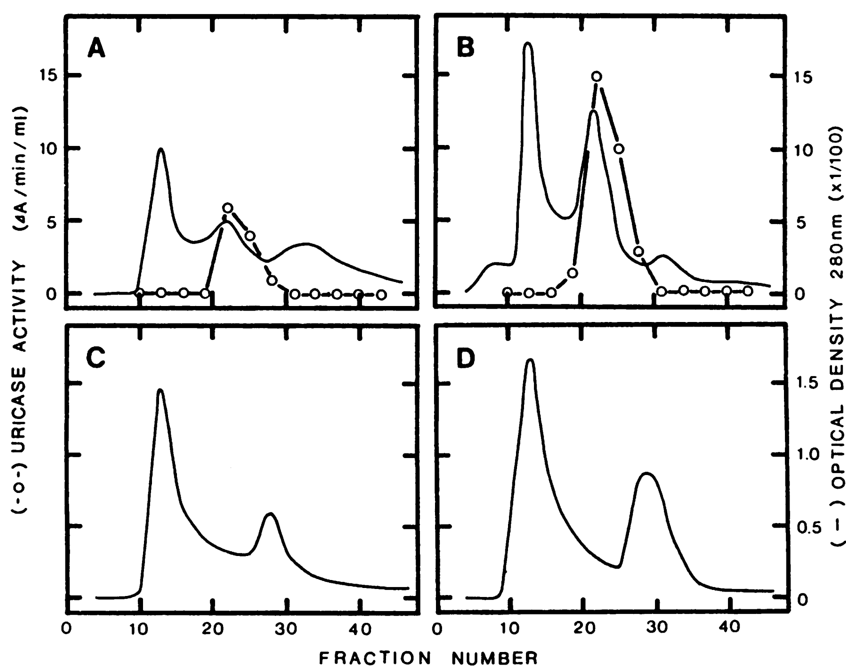

Figure 3. Native size of N-35 expressed in *E. coli* and its comparison with that expressed in soybean nodules. S-30 fractions (30 μg) from nodule (A) and *E. coli* carrying pHN35 (B), respectively, were loaded on a fast protein liquid chromatography Superose-12 gel filtration column with 100 mM of Tris-HCl buffer (pH 7.5) including 50 mM NaCl. The same fractions from nodule (C) and *E. coli* carrying pHN35 (D) were loaded onto the same column with 10 mM of phosphate buffer pH 7.5. The first peak (observed in the tenth fraction) showed the absorbance of blue dextran. Uricase activities were not detectable in C and D under these conditions.

is assembled as a tetrameric enzyme, we looked for whether N-35 expressed in *E. coli* cytoplasm is in fact assembled into a proper holoenzyme in the absence of peroxisomes. The DEAE unbound fraction was separated on gel-filtration chromatography to elucidate the native size of the N-35 made in *E. coli* and soybean nodules (Fig. 3, A and B). Both N-35 from nodules and *E. coli* were eluted as about 110-kD molecular mass, which agrees with the molecular mass of native enzyme (1). Although N-35 purified from nodules was eluted as a molecule of 110 kD in high salt conditions, it was eluted as a 35-kD species in lower salt conditions (Fig. 3C). The same phenomenon was also observed in N-35 from *E. coli* (Fig. 3D), suggesting that a noncovalent interaction is responsible for the assembly of this enzyme into a tetrameric form. This observation also indicates that N-35 expressed in *E. coli* forms a homotetramer in the same manner as in nodules.

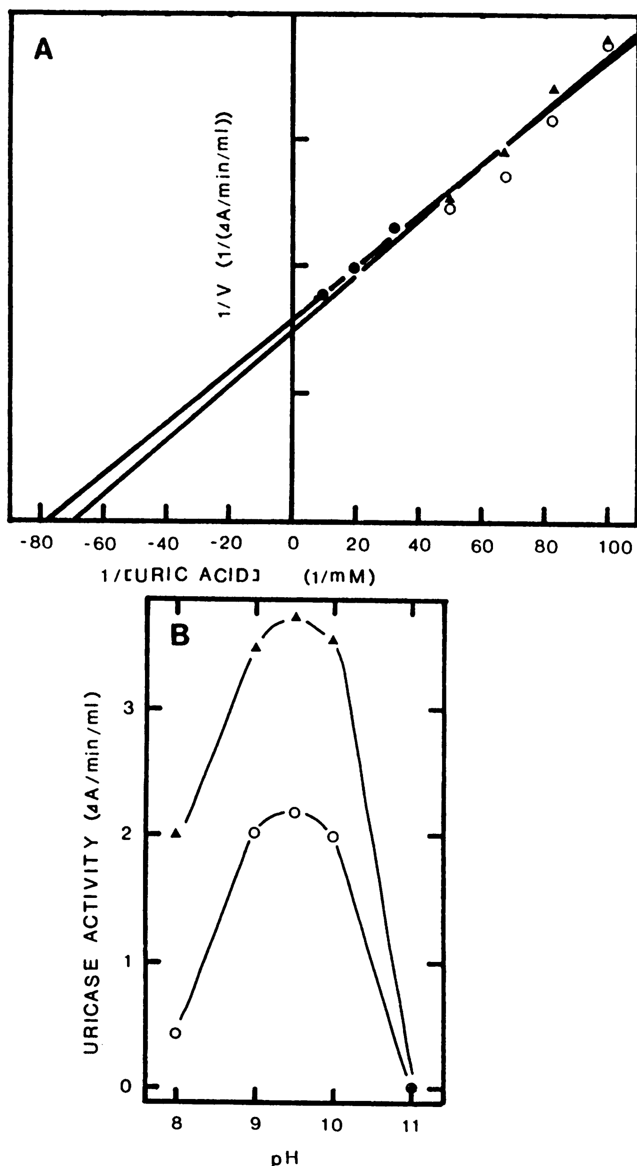

Figure 4. K_m and pH optima of N-35 expressed in *E. coli* and in nodules. A, Uric acid dependence of N-35 activity at pH 9.5. The K_m for uric acid: N-35 was determined according to the method of Lineweaver and Burk. B, pH optimum of N-35 expressed in *E. coli* and nodules. N-35 from nodules is indicated by open circular (○) and a solid triangle (▲) for N-35 from *E. coli*.

N-35 is translated on free polysomes and posttranslationally transferred into peroxisomes (1). Both N-35 purified from transformed bacteria and nodules showed almost the same apparent K_m (15 μM and 13 μM, respectively) which agrees with that reported by Lucas *et al.* (12) and also the same pH optima at 9.5 (Fig. 4). Both proteins showed identical specific activity (Table II). Correct folding and assembly of enzyme proteins are essential for functional activity in different cellular conditions. Folding and assembly are strongly affected by such cellular conditions as ions, oxidation conditions, and chaperones (13). These different conditions still allow the correct protein assembly of N-35 in *E. coli* without interfering with enzymatic activity. It seems that crossing of the peroxisomal membrane is not essential for the assembly of N-35. We constructed a N-35 gene derivative devoid of its 34 carboxyl-terminal amino acid sequence. Although purified N-35 is a very stable enzyme (1) this truncated protein seems to be unstable in *E. coli* and was not detected as a protein band on SDS-PAGE (data not shown). The instability of the truncated product and a lack of uricase activity detected in this construct imply the importance of the carboxyl terminus for stability and enzymatic activity of N-35.

A promoter fragment isolated from N-35 gene of soybean (16) was inserted into tobacco following a reporter gene (chloramphenicol acetyl transferase) fusion. However, we were not able to induce expression of this gene in tobacco, indicating that there are some specific requirements for nodule factors for the induction of this gene. When introduced into yeast (22), this gene functions constitutively, suggesting that a repressor mechanism may be operative in nonnodule plant tissue to control the expression of this gene. Because most of the nodulin gene promoters function only in the legume background, transformation of a ureide-producing legume (such as *Vigna*) with the N-35 gene may allow dissection of the *cis*-acting elements and *trans*-acting factor as well as metabolites necessary for the induction of this gene in root nodule.

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