Deoxyhypusine Synthase from Tobacco

cDNA ISOLATION, CHARACTERIZATION, AND BACTERIAL EXPRESSION OF AN ENZYME WITH EXTENDED SUBSTRATE SPECIFICITY*

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Deoxyhypusine synthase catalyzes the formation of a deoxyhypusine residue in the translation eukaryotic initiation factor 5A (eIF5A) precursor protein by transferring an aminobutyl moiety from spermidine onto a conserved lysine residue within the eIF5A polypeptide chain. This reaction commences the activation of the initiation factor in fungi and vertebrates. A mechanistically identical reaction is known in the biosynthetic pathway leading to pyrrolizidine alkaloids in plants. Deoxyhypusine synthase from tobacco was cloned and expressed in active form in Escherichia coli. It catalyzes the formation of a deoxyhypusine residue in the tobacco eIF5A substrate as shown by gas chromatography coupled with a mass spectrometer. The enzyme also accepts free putrescine as the aminobutyl acceptor, instead of lysine bound in the eIF5A polypeptide chain, yielding homospermidine. Conversely, it accepts homospermidine instead of spermidine as the aminobutyl donor, whereby the reactions with putrescine and homospermidine proceed at the same rate as those involving the authentic substrates. The conversion of deoxyhypusine synthase-catalyzed eIF5A deoxyhypusinylation pinpoints a function for spermidine in plant metabolism. Furthermore, and quite unexpectedly, the substrate spectrum of deoxyhypusine synthase hints at a biochemical basis behind the sparse and skew occurrence of both homospermidine and its pyrrolizidine derivatives across distantly related plant taxa.

The eukaryotic initiation factor 5A (eIF5A), a small 17.4-kDa protein, is activated by a post-translational modification of a specific lysine residue to hypusine [(N'-4-amino-2-hydroxybutyl)lysine] in an enzyme-catalyzed two-step mechanism (reviewed in Refs. 1 and 2). In the first step, the aminobutyl moiety of the polyamine spermidine is transferred by deoxyhypusine synthase (EC 1.1.1.249) in an NAD⁺-dependent reaction to the ε-amino group of a specific lysine residue in the eIF5A precursor protein to form deoxyhypusine. In the second step, deoxyhypusine hydroxylase (EC 1.14.99.29) catalyzes the hydroxylation of the deoxyhypusine residue to hypusine. Activated eIF5A is the only protein in which the unusual amino acid hypusine has been detected to date (3, 4), the modification is one of the most specific post-translational modifications known (5, 6).

eIF5A seems to be ubiquitous among eukaryotes (7) and archaebacteria (8). Its amino acid sequence is highly conserved, and the 12 amino acids surrounding the hypusine residue are identical in all eukaryotes studied. Although known for nearly 2 decades, the function of eIF5A is still obscure. Because of its in vitro activity in stimulating methionyl puromycin synthesis (9), eIF5A was classified as a protein synthesis initiation factor, though subsequent doubts have arisen as to whether initiation of protein synthesis is a major function of this protein (2). Using a yeast mutant, it was shown that depletion of eIF5A causes an immediate inhibition of cell growth but only a moderate inhibition (30%) of total protein synthesis (10). There is convincing evidence that post-translational hypusine synthesis is required for eIF5A activity and, as a consequence, for eukaryotic cell proliferation. Moreover, it confirms an essential function of spermidine.

Deoxyhypusine synthase has been purified from different eukaryotic species (rat testis (11), HeLa cells (12), Neurospora crassa (13), and yeast (14, 15)) and was cloned and overexpressed from human (16, 17) and N. crassa (18). CDNA or gene sequences for the protein have been identified in several other species including archaebacteria (19), but there are no data available about this enzyme in plants. Because deoxyhypusine synthase is highly conserved across eukaryotes and archaebacteria (20), and since hypusine-containing eIF5A proteins have been found in plants (21–24), the existence of the enzyme in plants seems likely.

Curiously, in the course of our research on the biosynthesis of pyrrolizidine alkaloids, a typical class of plant secondary compounds (27), we characterized an enzyme whose biochemical and molecular properties greatly resemble those of deoxyhypusine synthase. This enzyme, homospermidine synthase, catalyzes in an NAD⁺-dependent reaction the transfer of the aminobutyl moiety of spermidine to a primary amino group of one histidine residues at the C terminus; GC-MS, gas chromatography coupled with a mass spectrometer; PCR, polymerase chain reaction; pkat, pico-}

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank† [EPI Data Bank with accession number(s) AJ242017. ‡ To whom correspondence should be addressed: Inst. für Pharmazeutische Biologie, Technische Universität Braunschweig, Mendelssohnstr. 1, D-38106 Braunschweig, Germany. Tel.: 49-531-391-5681; Fax: 49-531-391-8104; E-mail: t.hartmann@tu-bs.de.

† The abbreviations used are: eIF5A, eukaryotic initiation factor 5A; eIF5A precursor protein from N. tabacum with six additional histidine residues at the C terminus; GC-MS, gas chromatography coupled with a mass spectrometer; PCR, polymerase chain reaction; pkat, pico-mole substrate per second (standard enzyme assay conditions).

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expression of functional deoxyhypusine synthase from tobacco, a plant that does not synthesize pyrroloidine alkaloids and is not known to produce homospermidine. We also demonstrate that tobacco deoxyhypusine synthase possesses homospermidine synthase activity.

**EXPERIMENTAL PROCEDURES**

Radiochemicals and Reagents—[1,4,14C]Putrescine (114 mCi/ml) and [14C]spermidine (N-3-aminopropyl)-(1,4,14C)tetramethylene-1,4-diamine (115 mCi/ml) were purchased from Amersham Pharmacia Biotech (Freiburg, Germany) and [14C]homospermidine (N-[1,4,14C]aminobuty1)-(1,4,14C)tetramethylene-1,4-diamine) was synthesized as described previously (29). Tag DNA polymerase and QIAEX II gel extraction kit were purchased from Qiagen (Hilden, Germany), primers P4-P11 were synthesized at MWG Biotech (Ebersberg, Germany), and endonucleases were obtained as follows: BamHI, Life Technologies, Inc.; NdeI, New England Biolabs; XhoI, Life Technologies, Inc.; NcoI, New England Biolabs; and Pfu DNA polymerase (Promega). The resulting DNA fragments and full-length clones were sequenced using the fluorescence dye terminator technology on an Applied Biosystems 377 DNA analyzer (Foster City, CA). DNA sequencing was performed by the DNA sequencing core facility at the University of Wisconsin-Madison.

**TABLE I Nucleotide sequence of primers used in PCR reactions**

| Primer | Nucleic acid sequence |
|--------|-----------------------|
| P1     | 5′-dGCG ARG AYT TYA THA ART GY 3′ |
| P2     | 5′-dCCTC TCR GGR KGN MR 3′ |
| P3     | 5′-dCCC CAN SWN ACN GCR TCR TC-3′ |
| P4     | 5′-dAAAT CTC ATA GTA TTA GGC TTA CAA GAA-GAA-3′ |
| P5     | 5′-dGGT GCA ATA GTA GAT-3′ |
| P6     | 5′-dTAAT CTT TAA TCT CCT CAT AAT TTT-3′ |
| P7     | 5′-dCAG TAG TTT CTA TTA GGA ACC AAT A-3′ |
| P8     | 5′-dGATATCAATAT GGA GAG GCC CTC AAC GT-3′ |
| P9     | 5′-dGATATCAATAT TAA AAC TTT GCA TCT TAT AIG GG-3′ |
| P10    | 5′-dGATATCAATAT TCG GAC GAA GAA CCA CAT-3′ |
| P11    | 5′-dTAATCTGAG CTT GGG GCC AAC GTC CTT GA-3′ |

**Amplification, Expression, and Purification of Tobacco Deoxyhypusine Synthase**—The supernatant of the sonicated cells was applied to a 25 x 5.0-cm DEAE-Fractogel column (Merck, Darmstadt) and eluted with a 40-ml linear gradient of 0–0.25 M NaCl in purification buffer at a flow rate of 2 ml/min. Fractions of 4.0 ml were collected. Fractions containing enzyme activity were pooled, adjusted to a NaCl concentration of 1.5 M, and applied to a phenyl-Sepharose CL-4B column (Amersham Pharmacia Biotech, 1.6 x 12 cm). For elution, in 10-ml fractions, a 120-mM linear gradient of 0.6–0 M NaCl in purification buffer that did contain only 5 mM KH2PO4, pH 9.0, 2 mM dithioerythritol, 0.5 mM NAD+, 0.1 mM EDTA and broken by sonication.

DNA Sequence Analysis—DNA fragments and full-length clones were sequenced using the fluorescence dye terminator technology on ABI Prism sequencers (SeqLab, Göttingen, Germany). The sequences of the fragments obtained by amplification using the degenerated primers and of the 3′ and 5′ end fragments were verified by sequencing the pETnDHS plasmid, which was amplified independently using the oligo(T) cDNA as template. DNA sequences were analyzed using the Wisconsin Sequence Analysis Package (version 8, Genetics Computer Group, Madison, WI).

Amplification, Expression, and Purification of Tobacco eIF5A Precursor Protein—Using sequence information from the eIF5A precursor protein cDNA of N. tabacum (22), two gene-specific primers, P10 (NdeI) and P11 (XhoI), were generated (Table 1). Amplification of the full-length eIF5A precursor protein cDNA was performed using the oligo(T) cDNA as template and Pfu DNA polymerase (Promega). The resulting 494-bp fragment was electrophoretically purified, NdeI/XhoI-digested and ligated into NdeI/XhoI-linearized pET-25b vector (Novagen), which contained the endonuclease recognition site for C-terminal His-tagging for metal chelate-affinity chromatography. The ligase constructions were transferred into XL1-blue cells (Stratagene), screened with primers P10 and P11 for the correct insert, and purified for sequencing and for transformation of E. coli BL21(DE3) (Stratagene). Resulting transformants were cultured at 37 °C in LB medium containing 50 μg/ml ampicillin overnight, transferred to fresh medium for an additional 1 h, and then induced with 1 mM isopropyl-β-D-thiogalactoside and grown for another 4 h. The cells were harvested vector (Novagen). After transformation of the ligated constructs into E. coli XL1-blue cells (Stratagene), positive clones were selected by PCR amplification using the primers P8 and P9. One clone was chosen, and the purified plasmid DNA was used for sequencing and for transformation of E. coli BL21(DE3) strain (Stratagene) for overexpression.

Expression of Tobacco Deoxyhypusine Synthase in E. coli—The pET-3a plasmid (31) containing the full-length deoxyhypusine synthase gene fragment pETnDHS was transformed into E. coli BL21(DE3). Ampicillin-resistant transformants were grown in LB medium containing 50 μg/ml ampicillin for approximately 20 h at 37 °C and, after induction with 1 mM isopropyl-β-D-thiogalactoside, for another 12 h. The harvested cells were frozen at −80 °C, then suspended in purification buffer (50 mM KH2PO4, pH 9.0, 2 mM dithioerythritol, 0.5 mM NAD+, 0.1 mM EDTA) and broken by sonication.
by centrifugation, resuspended in lysis buffer (50 mM NaH₂PO₄, 0.3 M NaCl, 5 mM 2-mercaptoethanol, 20 mM imidazole), and sonicated for 5 min. From the supernatant, the His-tagged protein (eifnt) was purified with nickel-nitrilotriacetic acid-agarose (Qiagen) according to the manufacturer’s instructions.

**Deoxyhypusine Synthase Assay**—The standard 50-μl assay contained 0.1 M glycine-NaOH buffer, pH 9.5, 1 mM dithiothreitol, 0.1 mM EDTA, 40 μM [14C]spermidine (0.06 μCi/assay), 40 μM eifnt precursor protein, 1 mM NAD⁺, and enzyme. Assays were incubated for 1–60 min at 30 °C. Reactions were stopped by adding 10 μl of 1 M potassium phosphate, pH 6.3, with 60 mM spermidine before they were adsorbed to a Whatman no. 3MM paper disc and developed as described elsewhere (14). If the reaction was stopped at different times for kinetic purposes, the reaction volume was scaled up according to the number of samplings.

**Homospermidine Synthase Assay**—Standard assays contained 1 mM dithiothreitol, 0.1 mM EDTA, 40 μM [1,4-14C]putrescine (0.06 μCi/assay), 40 μM spermidine, 1 mM NAD⁺, and enzyme. Incubations were done for 1–60 min at 30 °C. Formation of labeled homospermidine was followed quantitatively by radio-TLC or high performance liquid radiochromatography as described previously (25).

**Identification of Deoxyhypusine and sym-Homospermidine as Products of the Deoxyhypusine Synthase Reaction**—Reaction mixtures of 1 ml containing spermidine, eifnt (40 μM each), 0.5 mM NAD⁺, and 25 μg of deoxyhypusine synthase were incubated at 30 °C for 2 h. Using nickel-nitrilotriacetic acid-agarose (Qiagen), the unmodified and modified eifnt protein was recovered and hydrolyzed under nitrogen in 6 N HCl at 120 °C for 24 h. Deoxyhypusine was purified using Amberlite CG120 II resin (32), evaporated to dryness, and derivatized according to Ref. 33, but with methanol, 3 N HCl instead of n-butanol, 3 N HCl to derivatize the carboxyl group. The amino groups were derivatized with 3-fluoroacetic acid. GC-MS was performed using a Carlo Erba 5160 gas chromatograph equipped with a 30 m × 0.32-mm fused silica column (DB-1) under the following conditions: injector, 250 °C; split-ratio, 1:20; carrier gas, helium 0.75 bar. The capillary column was directly coupled to a Finnigan MAT 4515 quadrupole mass spectrometer. Electron impact-mass spectra were recorded at 40 eV. For identification of sym-homospermidine, an aliquot of the enzymatic reaction that contained 40 μM putrescine instead of eifnt was derivatized with methyl chlorofomate medium (34) and analyzed by GC-MS.

**RESULTS**

**Cloning and Identification of Tobacco DHS**—Reasoning that the amino acid sequence of plant deoxyhypusine synthase would be similarly conserved as its homologues from other sources, an alignment of deoxyhypusine synthase amino acid sequences from human, yeast, *N. crassa* (nc), *Saccharomyces cerevisiae* (sc), and *M. jannaschii* (mj) was used to design and construct the degenerate primers P1–P3 (Fig. 2). With the primer pair P1-P3, a 560 bp fragment could be amplified by reverse transcription-PCR with RNA isolated from a young leaf of *N. tabacum*. The fragment contained an open reading frame that showed high sequence similarity to deoxyhypusine synthases from other sources. This sequence information was used to...
The molecular mass of the native deoxyhypusine synthase was determined to be approximately 190 kDa by size exclusion chromatography on a Superdex 200 column (data not shown), suggesting that plant deoxyhypusine synthase, like the enzymes from the other sources, is a homotetramer.

**Identification of Reaction Products**—As substrate for the deoxyhypusine synthase assay, the eIF5A precursor protein of *N. tabacum* was applied. Using the known sequence (22), the precursor protein of tobacco was cloned into pET-23b and expressed in *E. coli* with an additional 6xHis tag at the C terminus. Sequence comparison of this clone (named eifnt) with the literature showed one synonymous nucleotide substitution at position 60 (T instead of C). Chemical identity of the reaction products of deoxyhypusine synthase as deoxyhypusine, homospermidine, and diaminopropane were confirmed by GC-MS. The mass spectrum of deoxyhypusine is shown in Fig. 5. To prove the specific labeling of the eIF5A precursor protein by deoxyhypusine synthase, a time-course experiment was performed in which enzyme assays containing 14C-labeled spermidine were analyzed by SDS-polyacrylamide gel electrophoresis. Fig. 6 shows that exclusively the eIF5A protein is labeled and the label increases in a time-dependent manner.

**Properties of the Recombinant Tobacco Deoxyhypusine Synthase**—The incorporation of radioactively labeled spermidine into the substrate protein was assayed using a filter paper assay. To ensure linearity of product formation, 50–100 μg aliquots were sampled at time intervals. The samples were frozen immediately and developed simultaneously. Pure tobacco deoxyhypusine synthase so expressed showed an activity of 350 pkat/mg of protein.

With regard to the reaction mechanism, deoxyhypusine synthase shows striking similarities to homospermidine synthase, the key enzyme in the biosynthesis of pyrrolizidine alkaloids (25, 26). The two enzymes transfer the aminobutyl group of spermidine either to the ε-amino group of a specific protein-bound lysine residue (deoxyhypusine synthase) or to a primary amino group of the diamine putrescine (homospermidine synthase) (Fig. 7). Both reactions are NAD+ dependent. The structural and kinetic similarities of the two enzymes prompted us to test whether the purified tobacco deoxyhypusine synthase accepts putrescine instead of the eIF5A precursor protein as substrate. The pure enzyme was incubated in the presence of 14C-labeled spermidine with equal (i.e., 40 μM) concentrations of eIF5A precursor protein and putrescine, respectively. The formation of the products were assayed as described under “Experimental Procedures.” In preliminary experiments it was proved that 40 μM spermidine is saturating in the assay with deoxyhypusine as product. The specific activity did not alter when raising the spermidine concentration up to 400 μM (Table
II). For putrescine a concentration of 40 \( \mu M \) is not saturating, but because the eIF5A precursor protein shows inhibition at concentrations above 40 \( \mu M \) substrate (Table II), both substrates were used in the assay at the same concentration of 40 \( \mu M \). The results are summarized in Table III. With the two different aminobutyl acceptors, specific activities of 150–350 pkat/mg were obtained, indicating that the two substrates are accepted at the same concentration with almost the same specific activity. To test whether spermidine as aminobutyl donor can be substituted by its homologue homospermidine, assays were performed in which \(^{14}C\)-labeled homospermidine was applied instead of labeled spermidine in the assay with eIF5A as the aminobutyl acceptor and non-labeled homospermidine together with \(^{14}C\)-labeled putrescine as the acceptor in the assay with homospermidine as the product (Table III). The results clearly show that homospermidine can substitute spermidine as the aminobutyl donor and that labeled putrescine is released instead of diaminopropane (Fig. 7). In the assays with deoxyhypusine as the product; the aminobutyl transfer is catalyzed with specific activities of 56 pkat/mg aminobutyl donor homospermidine and 152 and 352 pkat/mg (donor spermidine) (Table III). In the assay with homospermidine as the product, the aminobutyl group of non-labeled homospermidine is transferred to labeled putrescine, forming labeled homospermidine and unlabeled putrescine with almost the same specific activity as with spermidine (Table III). We discriminated between homospermidine supplied as substrate and that resulting as the product by feeding \([^{14}C]\)putrescine and \([^{12}C]\)homospermidine in the assay and detection of the resulting \([^{14}C]\)homospermidine. Performing these assays, it was crucial to keep incubation times as short as possible to ensure linearity, because the resulting products, i.e. homospermidine and putrescine from spermidine) and 152 and 352 pkat/mg (donor spermidine) (Table III). In the assay with homospermidine as the product, the aminobutyl group of non-labeled homospermidine is transferred to labeled putrescine, forming labeled homospermidine and unlabeled putrescine with almost the same specific activity as with spermidine (Table III). We discriminated between homospermidine supplied as substrate and that resulting as the product by feeding \([^{14}C]\)putrescine and \([^{12}C]\)homospermidine in the assay and detection of the resulting \([^{14}C]\)homospermidine. Performing these assays, it was crucial to keep incubation times as short as possible to ensure linearity, because the resulting products, i.e. homospermidine and putrescine from spermidine) and 152 and 352 pkat/mg (donor spermidine) (Table III). 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homospermidine cleavage, act as substrates again and compete for the active site.

**DISCUSSION**

We have cloned and characterized deoxyhypusine synthase from a plant source. The cDNA insert in the plasmid pETnt-DHS derived from mRNA of young tobacco leaves encodes a protein of 379 amino acids that shows strong sequence conservation with deoxyhypusine synthase from other eukaryotic sources and archaea (Fig. 1). Not only the eIF5A is conserved in all eukaryotes but also the mechanism of its post-translational modification. It has already been shown that the eIF5A precursor protein isolated from a plant (i.e. alfalfa) can be activated by hypusine formation in yeast (35). The sequence of tobacco deoxyhypusine synthase shares all strictly conserved residues found in the primary structure of the enzymes from other sources (Fig. 1, including those that have been identified to be functionally important at the substrate and coenzyme binding area in the three-dimensional structure (36). Searching the GenBank and EMBL data base for proteins related to the amino acid sequence of the tobacco enzyme, only sequences of deoxyhypusine synthases are found.

Tobacco deoxyhypusine synthase is a tetramer composed of identical subunits. This is in agreement with the three-dimensional structure of the human enzyme, which is a tetramer composed of two tightly associated dimers containing a total of four active sites, two in each dimer interface (36). In each active site of the human enzyme, the catalytic portion is located on the amino acid sequence of the tobacco enzyme, only sequences of deoxyhypusine synthases are found.

Incorporation of radioactively labeled [14C]spermidine into eifnt-precursor protein by deoxyhypusine synthase activity. Of a standard deoxyhypusine synthase assay, 10-μl aliquots were taken after different incubation times and stopped by adding to 10 μl of boiling Laemmli’s stop solution. The samples were separated on a 15% SDS-polyacrylamide gel (58), blotted to a PVDF membrane, stained with Coomassie Blue (A), and exposed to Kodak XAR 5 film for 5 days (B); 10-kDa protein ladder (Life Technologies, lanes 1 and 8, the 50-kDa band is indicated by an arrowhead; lane 8 was copied from A to B for better orientation), enzyme assay aliquots after 0, 1, 2, 4, 8, and 16 min incubation time (lanes 2–7). The two predominant bands represent deoxyhypusine synthase and eIF5A-precursor protein (45 and 20 kDa, respectively).

**TABLE II**

| Assay | Spermidine | eifnt | Putrescine | Specific activity |
|-------|------------|-------|------------|-----------------|
|       | μM         | μM    | μM         | phat/μg         |
| 1     | 40         | 40    | 40         | 234             |
| 2     | 400        | 40    | 40         | 232             |
| 3     | 40         | 100   | 40         | 132             |
| 4     | 40         | 40    | 40         | 250             |
| 5     | 400        | 40    | 40         | 292             |
| 6     | 40         | 400   | 40         | 1283            |

Putrescine as substrate with the same activity as its authentic substrate. Obviously, free putrescine fits into the active site in the same manner as the specific protein-bound lysine residue of eIF5A. The free amino acid lysine is not accepted as substrate (1). It seems likely that deoxyhypusine synthases from other sources may also accept free putrescine as substrate, but this awaits experimental confirmation. The aminobutylation of the eIF5A precursor protein has previously been thought to be a highly specific reaction. A similar situation exists with the aminobutyloyl donor spermidine, which can be replaced by its
homologue homospermidine in the reaction catalyzed by the tobacco enzyme (Table III). Again, homospermidine is used almost as efficiently as the genuine substrate. Homospermidine has not previously been recognized as substrate, but it was shown to inhibit deoxyhypusine synthase activity (38) presumably by competing with spermidine. The only homologue of spermidine that has been shown to function as substrate of the enzyme from rat testis was aminopropylcadaverine (39).

A peculiar property of deoxyhypusine synthase seems to be its low specific activity. The values obtained for the tobacco enzyme with the different substrates range from 56 to 352 pkat/mg. This corresponds to turnover numbers \( k_{\text{cat}} \) of \( 2.4 \times 10^{-2} \text{ s}^{-1} \) to \( 1.5 \times 10^{-2} \text{ s}^{-1} \). These values agree with the corresponding values calculated from reference data of deoxyhypusine synthase purified from other sources: human, \( k_{\text{cat}} \) 1.0 \( \times \) \( 10^{-2} \text{ s}^{-1} \) (16); N. crassa, 7.0 \( \times \) \( 10^{-4} \text{ s}^{-1} \) (13); rat testes, 9.6 \( \times \) \( 10^{-2} \text{ s}^{-1} \) (40); Saccharomyces carlsbergensis, 2.1 \( \times \) \( 10^{-2} \text{ s}^{-1} \) (15). The low turnover number appears not to be related to the protein-protein interaction during enzyme catalysis of deoxyhypusine synthase because putrescine is turned over at a comparable rate (Table III).

The efficient aminobutylation of putrescine by tobacco deoxyhypusine synthase raises the question about the role of homospermidine, the product of this side activity. Homospermidine is one of the so-called “uncommon polyamines,” which occasionally accompany ubiquitously distributed amines such as putrescine, spermidine, and spermine. Homospermidine has been sporadically found in eubacteria (41), archaeabacteria (42), and eukaryotes (43–46). In eubacteria, homospermidine is synthesized by bacterial homospermidine synthase (EC 2.5.1.44) through a reaction which is quite similar to that catalyzed by deoxyhypusine synthase or plant homospermidine synthase. However, bacterial homospermidine synthase, which has been cloned and characterized (47), has no structural similarity to deoxyhypusine synthase. Deoxyhypusine synthase seems to be conserved in all archaeabacteria and eukaryotes. Hence, it can be argued that this enzyme may be responsible for the occurrence of homospermidine in these organisms. Of course, it is necessary to confirm whether deoxyhypusine synthase from other organisms show the same substrate specificity as the tobacco enzyme, i.e. whether they possess the ability to synthesize homospermidine. Indirect support for this notion comes from the observation that in the few animal species in which homospermidine has been detected, this polyamine was always found in tissues with high metabolic or cell growth activity, e.g. Syrian hamster epididymis (48) and testes, ovaries, and spleen of the Japanese newt (49). These are precisely such actively proliferating tissues in which high activities of hypusine formation have been detected (e.g. testes and Chinese hamster ovary cells) (1, 50). With respect to the ubiquitous occurrence of putrescine (51), it is still unresolved whether homospermidine is synthesized in vivo in tobacco without being accumulated in detectable amounts or whether the formation of homospermidine is the result of indiscriminate enzyme activity detectable only in vitro. The occurrence of homospermidine has not been reported for tobacco, although this plant is rather frequently used in polyamine research (52–54). However, a recent GC-MS analysis of the polyamine fraction of young tobacco leaves revealed very small but unambiguously detectable amounts of homospermidine. Only a few plants are known to produce large quantities of homospermidine (45, 46). Even in plants producing pyrrolizidine alkaloids, however, where homospermidine is the first pathway-specific intermediate in the biosynthesis of these alkaloids, homospermidine is channeled so effectively into the alkaloid pathway that it is impossible to detect free homospermidine unless the successive step of the pathway is inhibited (25). Homospermidine synthase in these plants is performed by homospermidine synthase, an enzyme that shows substantial sequence similarities to deoxyhypusine synthase, but that is devoid of the activity to produce deoxyhypusine.

The existence of a highly conserved deoxyhypusine synthase in plants and its role in the similarly conserved process of deoxyhypusine formation strongly indicates an essential function of plant eIF5A. This function is still unknown. Among plant physiologists, the suspected but unknown roles of spermidine in plant growth and development are controversially discussed (52–55). The requirement of spermidine as an essential substrate for deoxyhypusine formation provides direct evidence for a function of this polyamine in plant metabolism.

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