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Identification of Posttranslationally Modified 18-Kilodalton Protein from Rice as Eukaryotic Translation Initiation Factor 5A[1]

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Using anther-derived rice (*Oryza sativa* L.) cell-suspension cultures, we have identified an 18-kD protein that is posttranslationally modified by spermidine and is influenced by endogenous polyamine levels. The posttranslationally modified residue has been identified as the unusual amino acid hypusine [N$^{\epsilon}$-(4-amino-2-hydroxybutyl)lysine] by reverse-phase high-performance liquid chromatography and gas chromatography-mass-spectrometry analyses. Differential labeling of the protein with labeled amines provided evidence that the butylamine moiety of spermidine is the immediate precursor of the hypusine residue in the protein. The eukaryotic translation initiation factor 5A (eIF-5A) is the only known mammalian protein that undergoes a similar posttranslational modification with hypusine. The purified 18-kD protein co-electrophoreses with human translational initiation factor eIF-5A in both isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gels. The purified protein from rice stimulated methionyl-puromycin synthesis in vitro, indicating its functional similarity to mammalian eIF-5A. The results presented provide evidence that the posttranslationally modified 18-kD protein from rice containing hypusine is eIF-5A and suggest the conservation of hypusine-containing translation initiation factor eIF-5A in eukaryotes.

Polyamines are ubiquitous in living cells. In plants they appear to be involved in and may play a regulatory role in such basic processes as cell division, morphogenesis, senescence, and responses to environmental stress (Evans and Malmberg, 1989; Flores et al., 1989; Galston and Kaur-Sawhney, 1990). Several studies have provided evidence of a close relationship between increases in polyamine biosynthesis and content and the rate of cell proliferation (Heby and Persson, 1990). Although polyamines have been implicated in a number of cellular processes including DNA, RNA, and protein synthesis (Bachrach, 1973; Tabor and Tabor, 1984), the mechanism by which they may regulate cellular growth is still unclear. In these processes, polyamines not only regulate macromolecular synthesis but they also appear to increase the fidelity of transcriptional and translational events (Jelenc and Kurland, 1979). One mechanism by which such regulation might be achieved involves polyamine binding to specific regulatory proteins (Tabor and Tabor, 1984; Heby and Persson, 1990).

eIF-5A (previously named eIF-4D) is one of a number of protein factors that stimulate the initiation phase of protein synthesis (Moldave, 1985). The purified protein from mammalian cells is small (16–18 kD) and weakly acidic (pI 5.1–5.4). Its primary function is to facilitate ribosomal subunit joining and the positioning of initiator tRNA (Met-tRNA$_i$) for the synthesis of the first peptide bond (Safer, 1989). This reaction is usually studied in vitro by a model reaction, the synthesis of methionyl-puromycin (Merrick, 1979). No other functional assay for eIF-5A is available at present. eIF-5A is distinguished by the possession of a unique residue, hypusine [N$^{\epsilon}$-(4-amino-2-hydroxybutyl)lysine], formed posttranslationally by transfer of a butylamino group from spermidine to a specific Lys, followed by a hydroxylation reaction (Park et al., 1981; Cooper et al., 1983). The presence of the unusual amino acid hypusine in eIF-5A has been shown recently to be essential for its biological activity in eukaryotic cells (Park, 1989; Park et al., 1991; Schnier et al., 1991). However, the precise functional role of eIF-5A and its hypusine component are yet to be elucidated. By using radiolabeled spermidine, the presence of this protein, as well as the unique posttranslational modification, has been detected in many different tissues and cells of mammals (Gordon et al., 1987). The occurrence of a protein containing the unusual amino acid hypusine, or a unique posttranslational modification involving polyamines, or the identification of the eukaryotic initiation factor eIF-5A, have not been reported in plant cells. The eIF-5A protein and its hypusine component formed by the modification of Lys are highly conserved from yeast to humans (Gordon et al., 1987), suggesting an important role for this protein and its modification in the regulation of protein synthesis. Schnier et al. (1991) have recently shown that eIF-

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Abbreviations: eIF-5A, eukaryotic translation initiation factor 5A; MGBG, methylglyoxal bis(guanylhydrazone); pI, isoelectric point.

5A and its hypusine modification are essential for the growth and viability of yeast cells.

Using anther-derived rice (*Oryza sativa* L.) cell-suspension cultures, we have identified an 18-kD protein that is post-translationally modified by spermidine, and the modification appears to be influenced by endogenous polyamine levels (Mehta et al., 1991a). We now identify the modified residue as the unusual amino acid hypusine and demonstrate a direct precursorial role for spermidine in its biosynthesis. In addition, we demonstrate that hypusine occurs predominantly in only one protein in rice suspension-culture cells. The purified 18-kD hypusine-modified protein was biochemically as well as functionally similar to mammalian eIF-5A. These results provide strong evidence for the 18-kD protein to be the eIF-5A from rice.

MATERIALS AND METHODS

Plant Material

Rice (*Oryza sativa* L.) cell-suspension culture of the Lys-rich mutant 4C was maintained in Murashige and Skoog (1962) medium supplemented with 1 mg/L 2,4-D and 2% Suc as a carbon source. The cells were grown on a gyratory shaker (New Brunswick Scientific, Edison, NJ) at 107 ± 2 rpm under continuous light (15 μmol m^{-2} s^{-1}) at 27°C.

Identification of the Modified Amino Acid Residue

For specific labeling of protein, rice cell-suspension cultures were incubated in the presence of 5 mM α-difluoromethylarginine and α-difluoromethylornithine for 12 h followed by incubation for 24 h in the presence of [terminal methylenes-^{3}H]spermidine.3HCl or [2,3-^{3}H(N)]-putrescine.2HCl (>15 Ci/mmol, New England Nuclear) with and without 10 μg/mL cycloheximide or 20 μM MGBG. Radiolabeled cells were harvested, homogenized, and analyzed by one- and two-dimensional gel electrophoresis as previously described (Mehta et al., 1991a, 1991b). The 18-kD protein band from one-dimensional polyacrylamide gels, as well as the single spot from two-dimensional gel electrophoresis, were excised and analyzed for the modified amino acid residue. Acid hydrolysis was performed in 6 M HCl at 110°C for 18 h in sealed tubes evacuated under nitrogen. Each hydrolyzate was centrifuged at 1000*g* for 5 min and passed through a 0.8-μm filter (Rainin, Woburn, MA). The filtrate was dried under vacuum in a Speed-Vac (Savant Instruments, Farmingdale, NY), the residue was resuspended in 250 μL of water and applied to a 0.8 × 7.5 cm column of Dowex 50 X_2 (NH_4^+ form, 200–400 mesh). The column was washed with 25 mL of water. Most of the commonly occurring amino acids were eluted with 20 mL of 1 M NH_4OH. The modified radiolabeled amino acid residue and standard hypusine, along with trace amounts of other amino acids, were eluted with 5 M NH_4OH (15 mL). This eluate was taken to dryness under vacuum and the residue was dissolved in water. Dansylation was conducted on aliquots of these solutions as described previously (Mehta et al., 1991a). The samples were filtered through a nylon 66 membrane (pore size 0.22 μm; Rainin) prior to HPLC analyses.

HPLC of the Purified Amino Acid Residue

The HPLC was carried out as previously described (Saftner and Baldi, 1990). The identities of the dansylated amino acids and hypusine were determined by retention times relative to known standards in the case of amino acids and chemically synthesized HPLC-purified hypusine for standard hypusine (Park et al., 1986). Authenticities of all the amino acids and hypusine were verified by GC-MS analysis of HPLC-eluting peak fractions. For measurement of the radioactivity, HPLC fractions were collected and the radioactivity was quantified in each fraction by liquid scintillation spectroscopy, using the Beckman Instruments quench monitoring (H#) technique. The identity of hypusine was determined by mixing the dansyl derivative of hypusine with dansylated samples of HPLC fractions and running the resulting mixture on HPLC using the same column and solvent gradient as for nondansylated samples. Hypusine derivatives were detected by an on-line fluorescence spectrophotometer as described previously (Mehta et al., 1991a).

GC-MS Analyses

GC-MS analyses of standard hypusine and the HPLC-purified modified amino acid residue co-migrating with hypusine under identical chromatographic conditions were performed with a Hewlett-Packard 5890A gas chromatograph connected to a 5970B mass selective detector and equipped with an HP 9133 computer. Ten microliters of HPLC-purified peaks of modified amino acid were transferred to 100-μL glass tubes held in larger glass-capped vials. For methylation of the dansylated amino acids, 10 μL of methylating agent (Methyl-8, dimethyl acetal; Pierce, Rockford, IL) was added to the samples and the reaction mixture was heated at 100°C for 30 min. Derivatized samples (1–5 μL) were injected in split mode (50–1) onto a bonded methyl silicone capillary column (HP-1, 23 mm × 0.2 mm). The carrier gas (He) flow rate was 30 cm s^{-1}. The optimum temperatures at a given flow rate and solvent composition were determined by making multiple injections at various temperatures. The temperatures giving large molecular intensities, without diminishing overall sensitivity, were utilized. After injection the operating temperature conditions were as follows. For the methyl derivatives of dansylated amino acids, initial temperature 110°C (isothermal for 4 min), 30°C min^{-1} to 240°C, 10°C min^{-1} to 300°C, and held at 300°C for 10 min. In all cases the temperature of the injector was 250°C and that of the detector and transfer line was 280°C. The ionizing energy was 70 eV for all runs. The ions were scanned from 50 to 500 mass units.

Preparation of the Radioactive Tracer Protein

Preparation from suspension-culture cells of a protein fraction containing the radiolabeled 18-kD protein for use as a tracer in the purification was carried out essentially as described by Mehta et al. (1991a). The 40 to 75% ammonium sulfate fraction employed as the tracer was found to contain essentially all of its radioactivity in the 18-kD protein.

Purification of the 18-kD Protein

The proteins in the cell-suspension culture homogenates were mixed with radioactive tracer 18-kD protein prepared from cultured cells of rice cell suspensions. The amount of the 18-kD protein added as a tracer (about 2.0 nmol, approximately 10^4 cpm) provided only a small contribution to the total amount of the 18-kD protein (between 250 and 275 nmol). The proteins were precipitated with ammonium sulfate and sequentially chromatographed on Mono Q (gradient 0–0.5 M KCl), Superose-12, and Mono S (batch elution, 0.5 M KCl) columns using the fast protein liquid chromatography system (Pharmacia). The efficiency and yield of the 18-kD protein at each step of purification was estimated by fluorometric measurement and scintillation counting. Based on one- and two-dimensional electrophoretic analyses of the purified protein, the 18-kD protein was judged to be apparently homogeneous.

Two-Dimensional Gel Electrophoresis

Samples containing 15 to 25 μg of protein were separated by two-dimensional gel electrophoresis as described previously (Mehta et al., 1991b). Proteins fractionated on polyacrylamide gels were either silver stained or visualized by fluorography. Silver-stained protein patterns revealed no evidence of carbamylation artifacts in polyacrylamide gels.

Analysis of the 18-kD Protein

Purified 18-kD protein (see Fig. 3b) was subjected to standard amino acid analysis using the Waters PicoTag system (Waters, Milford, MA). N-terminal sequencing was carried out by automated amino acid sequencing using a model 477A protein microsequencer with an on-line 120A PTH analyzer (Applied Biosystems, Foster City, CA). Chemicals for sequencing and analysis of data were as described by the manufacturer. The average repetitive yields for amino acids were more than 90%.

Methionyl-Puromycin Assay

The activity of eIF-5A in purified 18-kD protein in stimulating methionyl-puromycin synthesis was determined and analyzed as described by Smit-McBride et al. (1989a), with minor modifications. Purified rice 18-kD protein (0–1 μg of protein) was tested for stimulation of methionyl-puromycin synthesis in a reaction volume of 30 μL containing 20 mM Tris-HCl, pH 7.6, 2 mM magnesium acetate, 75 mM KCl, 10 μM 2-mercaptoethanol, 2 pmol of [^{3}H]Met-tRNA$_i$ (specific activity 3.75×10^4 cpm/pmol of Met), 0.8 mM GTP, 1 mM puromycin, 0.5 mM initiation codon AUG, 0.1 A_{260} unit of 40S ribosomal subunits, 0.25 A_{260} unit of 60S ribosomal subunits, and a mixture (10 μg of protein) of partially purified initiation factors.

RESULTS

Identification of Spermidine as the Immediate Precursor of the Modified Amino Acid Moiety in the 18-kD Protein

Good labeling of an 18-kD protein was observed with [2,3-^{3}H]putrescine, from which spermidine and spermine would be labeled in their butylamine moiety (Tabor and Tabor, 1984), and with [methylenes-^{3}H]spermidine, in which both butylamine and propylamine positions are labeled (Table I). In the presence of MGBG, a potent inhibitor of the enzyme *S*-adenosylmethionine decarboxylase and hence spermidine synthesis (Tabor and Tabor, 1984), little label was incorporated into the 18-kD protein from putrescine (Table I). In contrast, addition of MGBG with labeled spermidine did not reduce labeling of the 18-kD protein. Since MGBG also inhibits conversion of spermidine to spermine (Tabor and Tabor, 1984), labeled spermine was not synthesized and did not affect labeling of the 18-kD protein. The relationship between the labeled spermidine and the labeled 18-kD protein indicates that the modification of the protein derives directly from cellular spermidine.

Additional experiments were conducted to clarify the role of modified amino acid residue turnover on preformed 18-kD protein. In the presence of cycloheximide, an inhibitor of protein synthesis (Mattoo et al., 1979), the incorporation of label from spermidine was reduced to undetectable levels (Table II), confirming our earlier results suggesting posttranslational modification of the 18-kD protein (Mehta et al., 1991a). The modified protein, once synthesized, is stable, since turnover of preformed modified 18-kD protein (or the modified amino acid moiety thereof) is not observed in the presence of cycloheximide (Table II).

Identification of the Radiolabeled Amino Acid Moiety as the Unusual Amino Acid Hypusine

Since the only known posttranslational modification of a protein involving spermidine is the synthesis of the amino acid hypusine, it was important to determine if the radiolabel on the ^{3}H-labeled 18-kD protein was indeed [^{3}H]hypusine. Therefore, the labeled protein band was acid hydrolyzed, and the labeled amino acid residue was purified by ion-exchange chromatography, dansylated, and further purified by reverse-phase HPLC as described in "Materials and Methods." Parallel analyses were performed on dansylated putrescine, spermidine, spermine, Lys, Arg, Met, hypusine, and deoxyhypusine. The major amount of radioactivity with coincident fluorescence co-eluted with standard, unlabeled dansyl hypusine (Fig. 1, A and B). A parallel analysis of purified

Table I. *Effect of MGBG on the incorporation of radioactivity from putrescine and spermidine into the 18-kD protein*

Cells were cultured for 24 h in the presence of α-difluoromethylarginine and α-difluoromethylornithine and labeled with amine, with or without 20 μM MGBG. Cells were harvested, washed with buffer, and homogenized, and total proteins were precipitated with TCA. The proteins were fractionated on a 12.5% polyacrylamide gel and radioactivity in the 18-kD protein was estimated as previously described (Mehta et al., 1991a).

Amine	MGBG	cpm in 18-kD Protein
[2,3-^{3}H]Putrescine	−	1000 ± 23
[2,3-^{3}H]Putrescine	+	40 ± 14
[terminal methylenes-^{3}H]Spermidine	−	600 ± 16
[terminal methylenes-^{3}H]Spermidine	+	800 ± 32

Table II. *Exchange and turnover of label in the 18-kD protein*

Rice suspension culture cells were labeled with [terminal methylenes-^{3}H]spermidine. Cycloheximide was added at 10 μg/mL where indicated. Protein extracts were analyzed by IEF/SDS-PAGE and the radioactivity in the 18-kD protein was determined as previously described (Mehta et al., 1991a).

Labeling Conditions	cpm in the 18-kD Protein at	
	12 h	24 h
−Cycloheximide	1137 ± 30	1511 ± 15
+Cycloheximide	38 ± 17	30 ± 5
−Cycloheximide, chased for 12 h in cycloheximide-containing medium	1005 ± 5	1150 ± 12

Glc-6-P dehydrogenase (Fig. 1C) failed to show any amino acid eluting at the position of hypusine.

In the chromatographic system used for hypusine detection, in which Arg was well resolved from other amino acids, the relative specific fluorescence of Arg was 70% of the value of hypusine. The recovery of Arg from eIF-5A was 70% due to the loss of Arg by hydrolysis and the detection method used. Since the number of Arg residues in eIF-5A is known, the hypusine content was estimated considering this difference. This estimation is further supported by the data presented in Table III. When allowance was made for this difference, the data indicate that eIF-5A contains approximately one hypusine residue per five Arg residues. The 18-kD protein contains approximately five Arg residues per molecule (Table III). Thus, the 18-kD protein contains approximately one hypusine residue per molecule.

Further evidence for the identity of this component was obtained from GC-MS analysis of the HPLC-purified modified amino acid residue and standard hypusine. The mass spectra obtained for the modified amino acid residue and standard hypusine were similar (Fig. 2, A and B) as well as similar to the mass spectra observed for hypusine isolated from human eIF-5A (Park et al., 1986).

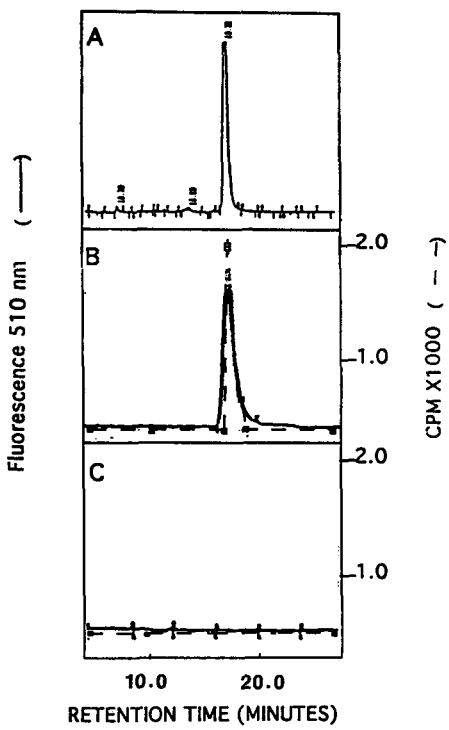

Figure 1. Chromatographic detection of hypusine in acid hydrolysates of purified 18-kD protein and Glc-6-P dehydrogenase. Purified 18-kD protein and Glc-6-P dehydrogenase (2 and 5 μg, respectively) were hydrolyzed with acid and analyzed by fluorometric and radiomatic detection as described in "Materials and Methods." Traces of fluorescence, radioactivity, and retention times during HPLC separation are presented for standard hypusine (A), 18-kD protein (B), and Glc-6-P dehydrogenase (C).

Two-Dimensional Electrophoretic Analysis

Rapidly growing rice suspension-culture cells were labeled with [methylenes-^{3}H]spermidine, and total cytoplasmic proteins were analyzed by two-dimensional gel electrophoresis (IEF/SDS-PAGE). Comparison of silver-stained gels permitted localization of the initiation factor, and superimposition of autoradiographs permitted determination of the correspondence of mobility between the 18-kD protein and human eIF-5A (Fig. 3). The 18-kD protein can be visualized on fluorographs of [^{3}H]spermidine-labeled preparations as the only significantly radiolabeled protein (Fig. 3C). When electrophoresed together, the stained human eIF-5A and the 18-

Table III. *Amino acid composition of the 18-kD protein*

Amino acid composition of the 18-kD protein from rice is compared to that from rabbit and human (lacking the initiator Met; Smit-McBride et al., 1989a).

Amino Acid Residues	Rice	Rabbit	Human	
	mol%	*mol%*	*mol%*[a]	*residues*[b]
Ala	7.0	8.4	7.8	12
Arg	3.4	3.2	3.3	5
Asn			2.6	4
Asn + Asp	10.4	11.0	11.1	17
Asp			8.5	13
Cys	2.4	2.9	2.6	4
Gln			3.3	5
Gln + Glu	8.9	7.7	11.1	17
Glu			7.8	12
Gly	9.8	9.6	9.2	14
His	2.3	2.6	2.6	4
Ile	5.8	7.5	7.2	11
Leu	10.1	9.0	8.5	13
Lys	6.1	8.1	8.5	13
Met	2.8	3.2	3.3	5
Pro	4.3	3.6	3.3	5
Ser	6.0	5.6	5.2	8
Phe	4.2	3.1	3.3	5
Thr	5.1	5.3	5.2	8
Tyr	6.3	1.9	2.0	3
Val	5.2	7.3	5.9	9
Trp	0.0	0.0	0.0	0

[a] Actual amino acid composition. [b] Number of residues per chain deduced from cDNA sequence.

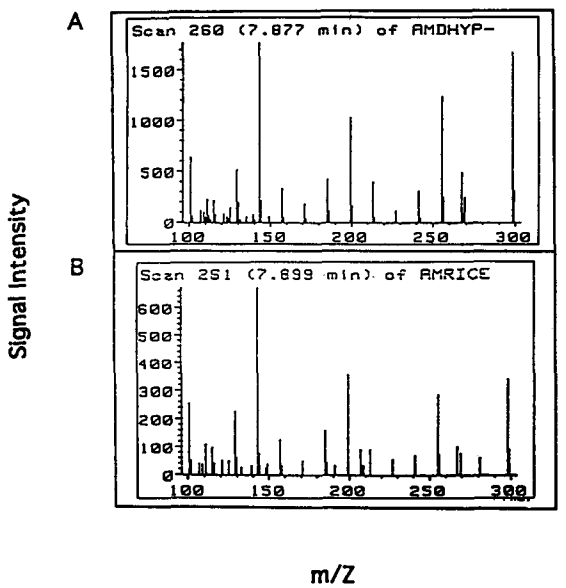

Figure 2. Mass spectra of methylated dansyl derivatives of standard hypusine (A) and the modified amino acid isolated from the purified 18-kD protein (B).

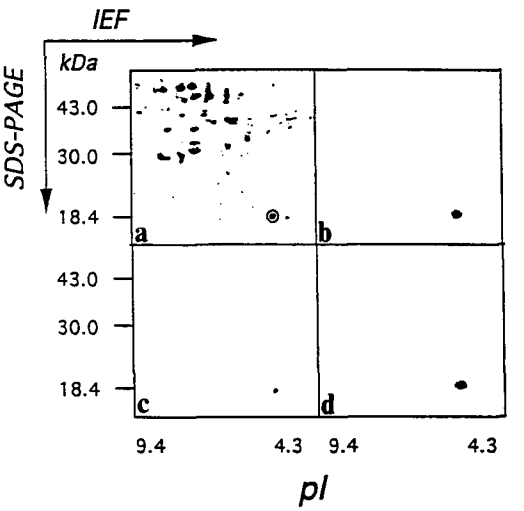

Figure 3. Two-dimensional polyacrylamide gel analysis of the 18-kD protein. Five to 25 μg of total cellular proteins (a), 2 μg of the purified 18-kD protein (b and c), and a mixture of 1 μg each of the purified 18-kD protein from rice and the human eIF-5A (d) were separated by IEF on gels containing pH 3 to 10 ampholytes followed by electrophoresis on 12.5% SDS-polyacrylamide gels as described previously (Mehta et al., 1991a). Portions of the silver-stained gels (a, b, and d) and fluorograph (c) surrounding the 18-kD protein are shown.

kD protein spot were indistinguishable (Fig. 3D), demonstrating identical electrophoretic mobility of human eIF-5A and the rice 18-kD protein. The tritiated proteins showed a single spot at 18-kD with a pI of 5.1.

Structural Analysis of the 18-kD Protein

The structure of the 18-kD protein was initially probed by automated Edman degradation of its amino terminus by analyzing over 250 pmol of protein purified from rice cell-suspension cultures. This procedure generated no useful sequence information, indicating that the amino terminus of the 18-kD protein is blocked. The amino acid composition determined for rice 18-kD protein closely matches the values calculated for rabbit eIF-5A and human protein lacking the amino-terminal Met (Table III). The purified human eIF-5A is relatively richer in acidic residues (25 Glu + Asp) than basic residues (22 Arg + Lys + His), consistent with its weakly acidic pI of 5.1. The presence of more acidic residues in the 18-kD protein is consistent with the weakly acidic nature of the rice eIF-5A, which is similar to other eukaryotic eIF-5A proteins. Translational initiation factors are known to be fairly conserved among different species (Gordon et al., 1987; Safer, 1989; Mehta et al., 1990). There are 46 hydrophobic residues, but the eIF-5A protein lacks Trp. The only substantial difference in amino acid composition between the rice protein and other eIF-5A proteins is in its Tyr content. The reason for this difference is unknown.

The 18-kD Protein Stimulates Methionyl-Puromycin Formation In Vitro

To obtain further evidence that the 18-kD protein purified from rice suspension-culture cells is the plant equivalent of eIF-5A, the purified 18-kD protein was tested for its activity in the methionyl-puromycin synthesis assay for eIF-5A (Smit-McBride et al., 1989b). The electrophoretically homogenous 18-kD protein stimulated the methionyl-puromycin synthesis over 4-fold when present at 1 μg in a standard reaction assay (Fig. 4). This result demonstrates that the 18-kD protein is functionally related to the eIF-5A protein reported from animal cells.

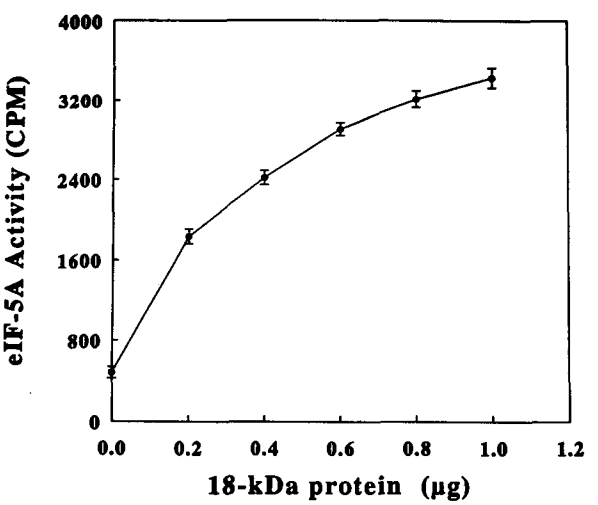

Figure 4. Stimulation of methionyl-puromycin synthesis by the 18-kD protein purified from rice suspension-culture cells. The details of the assay are described in "Materials and Methods." The eIF-5A activity is reported as cpm of [^{3}H]Met incorporated into methionyl-puromycin.

DISCUSSION

This paper describes the characterization of the spermidine-modified 18-kD protein from rice cell-suspension cultures. A number of observations provide strong evidence that the 18-kD protein from rice is the translational initiation factor eIF-5A, which contains the unusual amino acid hypusine. In yeast and animal cells, this amino acid is synthesized posttranslationally by the transfer of a butylamino group of spermidine to a Lys residue followed by a hydroxylation reaction (Cooper et al., 1983; Park et al., 1986). In rice cells, radioactivity was incorporated by posttranslational modification from [methylenes-^{3}H]spermidine and became covalently associated with an 18-kD protein in an acid-stable fashion. Once modified, the protein is stable and does not turn over within 12 h. There has been an accumulation of evidence for posttranslational modifications of cellular proteins involving the ϵ-amino groups of Lys residues (Sitaramayya et al., 1980). Small amounts of labeled putrescine and spermidine have been shown to bind covalently to proteins through a γ-glutamylamide linkage (Folk et al., 1980; Apelbaum et al., 1988). However, the acid stability of the modified amino acid residue in the 18-kD protein from rice and eIF-5A from animal tissues indicates that the attachment of label is not by an acid-labile γ-glutamylamide linkage. The relationship between the labeled amines used in labeling experiments, the composition of the cellular amine pools with and without polyamine synthesis inhibitors, and the appearance of the label in the 18-kD protein provide strong evidence that the hypusine in the 18-kD protein derives directly from cellular spermidine.

The modified amino acid residue from the 18-kD protein of rice was identified by reverse-phase HPLC as well as GC-MS as hypusine. All of the radioactivity in this protein derived from [^{3}H]spermidine was recoverable as hypusine. Only one protein, the initiation factor eIF-5A, has been shown to contain this unusual amino acid in all eukaryotic cells examined thus far (Park et al., 1981; Cooper et al., 1983; Gordon et al., 1987; Sandholzer et al., 1989; Mehta et al., 1990; Schnier et al., 1991). Analysis of purified eIF-5A from rice revealed that it contains approximately 1 mol of hypusine per mol of protein.

The 18-kD protein has electrophoretic properties in both IEF and SDS-PAGE identical to those of initiation factor eIF-5A from human peripheral lymphocytes (Smit-McBride et al., 1989b). The identical two-dimensional gel electrophoretic mobility of eIF-5A protein of rice and human reported here extends similar findings using eIF-5A from rabbit (Smit-McBride et al., 1989a) and Chinese hamster (Huang et al., 1987) and is further evidence of the highly conserved structure of this protein from plant as well as animal tissues (Gordon et al., 1987; Huang et al., 1987). Two-dimensional gel analysis of the spermidine-labeled cells detected two protein spots on silver staining, and one radioactive spot, which had identical mol wt but different pI values, and these have been observed in organisms from yeast to mammals (Gordon et al., 1987). On this basis it has been suggested that the two silver-stained proteins may represent modified (stained and labeled) and unmodified (stained) forms of eIF-5A (Gordon et al., 1987). In rice, the modified form (spot circled in Fig. 3a) appears to be more abundant than the unmodified form (spot next to the circled spot in Fig. 3a).

The amino acid composition of the 18-kD protein reported here from rice is very similar to the eIF-5A reported from human, rabbit, and yeast (Smit-McBride et al., 1989a). All of these proteins are slightly richer in acidic amino acids, have similar numbers of hydrophobic residues, and lack Trp. Because the amino terminus of the 18-kD protein appears to be blocked and no amino acid sequence information is available from this region, it is not known whether the initiator Met residue is present in the mature protein or is removed by amino-terminal Met aminopeptidase. It seems likely that Met is in fact cleaved from the 18-kD protein. The resulting amino-terminal residue may be acetylated, since this amino acid appears to be a favored substrate for the amino-terminal acetylase (Quigley et al., 1978).

A number of eukaryotic initiation factors have been shown to promote initiation of protein synthesis by transiently associating with ribosomes. The precise function of these factors has been elucidated primarily by in vitro assays with purified cell components (Safer, 1989). Major evidence for the function of eIF-5A in protein synthesis is provided by its role in stimulating the synthesis of methionyl-puromycin. The purified 18-kD protein from rice cells stimulated methionyl-puromycin synthesis. The activity of the 18-kD protein from rice suggests a role in the positioning of Met-tRNA$_i$ for the synthesis of the first peptide bond (Safer, 1989). The results demonstrate that the purified 18-kD protein from rice is functionally related to mammalian eIF-5A, and that this factor is conserved in all eukaryotes, including plants.

The roles of spermidine and other polyamines in cellular metabolism have been extensively studied (Evans and Malmberg, 1989; Flores et al., 1989; Galston and Kaur-Sawhney, 1990). Polyamines are known to bind to nucleic acids and to affect the process of protein synthesis in vitro and in vivo (Jelenc and Kurland, 1979; Tabor and Tabor, 1984). Yet, no precise functions for these small molecules have been identified. Since hypusine formation represents one of the most specific polyamine-dependent processes, it is tempting to speculate that some of the important actions of polyamines in growth regulation may be mediated via hypusine formation. This notion is strengthened by observations that hypusine formation increases after growth stimulation in mammalian cells (Park et al., 1981). The physiological significance of hypusine formation is further suggested by its highly conserved nature in yeast, insect (Gordon et al., 1987), and human cells, and plants. Our results indicate that an essential role for spermidine is to participate in the unique and necessary hypusine modification of eIF-5A. Experiments are in progress to determine how the hypusine modification may contribute to the essential function of eIF-5A in plant cells.

Since eIF-5A can be uniquely radiolabeled in its hypusine moiety, it can be easily identified in various subcellular locations by two-dimensional gel electrophoresis. This permits studies of relative synthesis and turnover rates in intact tissues, as well as interactions with other proteins. The present findings, along with the earlier knowledge of the mechanism of hypusine synthesis in mammalian cells, provide a firm basis upon which to conduct a search for the enzymes involved in this posttranslational event, and, subsequently,

an investigation of their cellular regulations. It is to be expected that such studies will lead to improved understanding of the role of this initiation factor in translation generally and in the regulation of differential protein synthesis during plant development and stress tolerance in which polyamines play a regulatory role.

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