Cloning and Expression of Human Deoxyhypusine Synthase cDNA

STRUCTURE-FUNCTION STUDIES WITH THE RECOMBINANT ENZYME AND MUTANT PROTEINS*

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Deoxyhypusine synthase catalyzes the first step in the post-translational formation of hypusine (N^4-(4-aminooxy-2-hydroxybutyl)lysine). cDNA clones encoding deoxyhypusine synthase were isolated from a human HeLa cell library. Full-length cDNA clones encoding a 369-amino acid protein (calculated molecular mass of 40,970 Da) and a shorter cDNA clone that would potentially encode a protein with an internal deletion of 56 amino acids (Asp<sup>262</sup>-Ser<sup>317</sup>) were isolated. The deduced amino acid sequence of the human enzyme shows a high degree of identity to that of yeast deoxyhypusine synthase and to the known sequences of tryptic peptides from the rat and Neurospora crassa enzymes. The recombinant enzyme formed upon expression in Escherichia coli effectively catalyzed deoxyhypusine synthase. Variant human recombinant proteins with (i) a truncation of 48 or 97 NH<sub>2</sub>-terminal amino acids, (ii) a truncation of 39 COOH-terminal amino acids, or (iii) an internal deletion (Asp<sup>262</sup>-Ser<sup>317</sup>) were inactive. A chimeric protein consisting of the complete human sequence and 16 amino acids of the yeast sequence (Gln<sup>197</sup>-Asn<sup>212</sup>, not present in the known enzyme) inserted between Glu<sup>193</sup> and Gln<sup>194</sup> exhibited moderate activity.

The unusual amino acid hypusine (N^4-(4-aminooxy-2-hydroxybutyl)lysine) is formed post-translationally in a single cellular protein, eukaryotic translation initiation factor 5A (eIF-5A)<sup>1</sup> (1–3). Hypusine biosynthesis involves two enzymatic steps. In the first step, deoxyhypusine synthase catalyzes the NAD-dependent transfer of the butylamin moiety of spermidine to the ε-amino group of a specific lysine residue of the eIF-5A precursor protein to form the intermediate deoxyhypusine (N^4-(aminobutyl)lysine) residue (1, 4–7). In the second step, mediated by deoxyhypusine hydroxylase, the conversion of the deoxyhypusine residue to the hypusine residue (8) completes eIF-5A maturation.

Hypusine is found in all eukaryotes and in some archaeabacteria, but not in eubacteria (1, 9). The amino acid sequence of the hypusine-containing protein, eIF-5A, is highly conserved, especially in the region surrounding the hypusine residue, suggesting an important fundamental function for this protein throughout eukaryotic evolution. In spite of its in vitro activity in the stimulation of methionyl puromycin synthesis and tentative identification as an initiation factor in eukaryotic protein synthesis (10, 11), its true cellular function is unknown (12, 13). However, hypusine and eIF-5A appear to be vital for cell proliferation in eukaryotes (12). The correlation between a reduction in hypusine formation and growth arrest in spermidine-depleted mammalian cells (14) and the requirements for expression of eIF-5A precursor protein and its hypusine modification for yeast viability (15, 16) strongly support this notion. Furthermore, inhibitors of the hypusine biosynthetic enzymes exert antiproliferative effects (17–19). In this regard, deoxyhypusine synthase presents a potential target for intervention in cell proliferation. A detailed knowledge of the amino acid sequence and properties of this enzyme should contribute to attaining more specific inhibitors of this enzyme and perhaps to better control of hyperproliferative diseases.

We have recently purified deoxyhypusine synthase from rat testis, determined the amino acid sequences of several tryptic peptides, and raised polyclonal antibodies to this enzyme (20). Cross-reactivity of anti-rat deoxyhypusine synthase with the human enzyme (from HeLa cells) was utilized to facilitate the cloning of cDNAs encoding human deoxyhypusine synthase.

In this study, we report the molecular cloning, expression, purification, and characterization of human recombinant deoxyhypusine synthase. Studies of deletion and insertion mutations, designed on the basis of a comparison of the amino acid sequences of the human and yeast (21) enzymes, provide insight into the structure-function relationship of the enzyme.

EXPERIMENTAL PROCEDURES

Materials

[1,8-^3H]Spermidine HCl (15 Ci/mmol) was purchased from DuPont NEN. Oligonucleotide primers were synthesized by the Midland Certified Reagent Co. The pET-11a expression vector and the host Escherichia coli B strain BL21(DE3) competent cells were from Novagen; T4 DNA ligase and the Superscript premplification system for single-strand cDNA synthesis were from Life Technologies, Inc.; AmpliTaq DNA polymerase was from Perkin-Elmer; restriction enzymes were from Boehringer Mannheim; and precast polyacrylamide gels and wide-range protein standards (Marker 12) were from Novex. ec-eIF-5A was purified from E. coli lysates after overexpression of the human eIF-5A cDNA as described previously (22). Total RNA from 144 JAR, a human trophoblast cell line (American Type Culture Collection), was kindly provided by Dr. Janet Kerr (NIDR) and total RNA from human epithelial cell lines by Dr. W. Andrew Yeudall (Laboratory of Cellular Development and Oncology, NIDR).

Methods

Deoxyhypusine Synthase Assay—The enzyme activity was measured as described previously (7, 20). A typical reaction mixture contained, in a total volume of 20 μl, 0.2 mM glycine NaOH buffer, pH 9.5, 1 mM dithiothreitol, 25 μg of bovine serum albumin, 1 mM NAD, 7 μM (2 μCi) [1,8-^3H]spermidine, 10 μg ec-eIF-5A, and enzyme. Incubations were at 37 °C for 60 min. The [^3H]deoxyhypusine formed was measured after its...
affinity adsorption to the immobilized antigen according to a published method as described previously (20). The polyclonal antibody was purified by sera against purified rat testis deoxyhypusine synthase were prepared by affinity chromatography as previously described (7, 23). One unit is defined as the amount of enzyme catalyzing the formation of 1 pmol/h of deoxyhypusine.

Immunopurification of Rabbit Polyclonal Antibodies—Rabbit antiserum against purified rat testis deoxyhypusine synthase was prepared by affinity adsorption to the immobilized antigen according to a published procedure (24). Briefly, rat testis enzyme and the amount of enzyme catalyzing the formation of 1 pmol/h of deoxyhypusine was separated by SDS-PAGE and then electrophoretically transferred to an Immobilon (polyvinylidene difluoride) membrane (Millipore Co., Bedford, MA). The 41-kDa enzyme band was excised, and the strip was incubated with antiserum. After washing the strip with 0.1 M glycine HCl buffer, pH 3.0, and immediately neutralized with 1.0 N NaOH.

Cloning and Sequencing of Human cDNAs Encoding Deoxyhypusine Synthase—Three immunopositive clones were detected. Nucleotide sequence analysis of their cDNA inserts was carried out by the dideoxy chain termination method (SequiTherm, Epicentre Technologies Corp.) and the cycle sequencing method (SequiTherm, Epicentre Technologies Corp.). One clone among these, designated hDS-1a, was identified as a tentative deoxyhypusine synthase clone based on the close similarity of its nucleotide sequence of clone hDS-13 (Fig. 1); these sequences numbered the basis of the nucleotide sequence of hDS-10, PCR primers (Pr5-1 and Pr3-2), and the cDNA insert (underlined in Table I) to facilitate cloning into the expression vector pET-11a.

Construction of a Human Deoxyhypusine Synthase Expression Plasmid—Full-length recombinant enzyme was produced by PCR amplification of the complete coding sequence and its expression in E. coli. On the basis of the nucleotide sequence of hDS-10, PCR primers (Pr5-1 and Pr3-2; see Tables I and II) representing the 5'- and 3'-end regions of the open reading frame were synthesized with a built-in NdeI site (5'-end primer) and a BamHI site (3'-end primer) and a BamHI site (3'-end primer) underlined in Table I) to facilitate cloning into the expression vector pET-11a. PCR was performed in a thermal cycler with a Perkin-Elmer PCR kit using hDS-10 plasmid DNA as template. The conditions for PCR were as follows: 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 35 cycles and a final extension reaction at 74°C for 6 min. The PCR product was digested with NdeI and BamHI, ligated to linearized pET-11a, and used to transform E. coli BL21(DE3) cells. The selected transformants were grown in LB medium supplemented with 50 μg/ml ampicillin. When the cell density reached an optical density of 0.6 at 600 nm, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and the cells were harvested by centrifugation 4 h later.

Construction of Deletion or Insertion Mutant Subclones of Human Deoxyhypusine Synthase cDNA and Their Expression in E. coli—Deletion and insertion mutants of human deoxyhypusine synthase cDNA were generated by a method similar to that used for the full-length enzyme. The nucleotide sequences of all the primers are listed in Table I; primer pairs and templates used to construct each subclone are indicated in Table II. The mutant subclones with a truncated 5'-terminus, hDS-m4 and hDS-m5, were generated using 5'-end primers that hybridized to internal sites (the coding sequences for amino acids 49–56 and 98–105, respectively) and Pr3-1, and the subclone with a truncated 3'-terminus, hDS-m6, was generated using Pr5-1 and Pr3-2, as indicated in Table II. A mutant cDNA with an internal deletion identical to that in hDS-1a was constructed by a two-step PCR procedure. In the first step, hDS-m1 and hDS-m2 cDNAs were generated in two separate reactions (Table II). The PCR products of the expected size were isolated from an agarose gel, and the mixture of the amplified fragments was used as template in the second PCR step to construct hDS-m3. The mutant subclone hDS-m3, with the insertion corresponding to Gin57→Asn121 of yeast deoxyhypusine synthase, was derived by a similar approach. In the first step, hDS-m7 and hDS-m8 cDNAs were generated in two separate reactions (Table II). The PCR products of the expected size were isolated from an agarose gel, and the mixture of the amplified fragments was used as template in the second PCR step to construct hDS-m9. The mutant subclone hDS-m9 was amplified using a mixture of hDS-m7 and hDS-m8 cDNAs as template. After cloning PCR products into pET-11a, the expression of altered recombinant proteins was carried out as described above.

Purification of Human Recombinant Deoxyhypusine Synthase—Iso-propyl-β-D-thiogalactopyranoside-induced E. coli cells (20 g) were resuspended in 120 ml of buffer A (50 mM Tris acetate, pH 6.8, 0.1 mM EDTA, 1 mM dithiothreitol, and 1 mM 4-(2-aminoethyl)benzensulfonyl fluoride) and sonicated for a 2-min interval at a setting of 70 watts, and the supernatant was collected after centrifugation for 30 min at 15,000 g. The supernatant was applied to a Fast Flow Q-Sepharose column (5 × 6 cm; Pharmacia Biotech Inc.) previously equilibrated with buffer A (50 mM Tris-acetate, pH 8.0; 0.5 mM KCl in buffer A). The fractions containing enzyme activity, which was eluted between 0.3 and 0.4 M KCl, were pooled and concentrated by precipitation with ammonium sulfate (40–80% saturation). After dialysis against buffer A, the enzyme fraction was applied to a Mono Q HR 10/10 column equilibrated with buffer A. The adsorbed enzyme was eluted with a 120-ml linear gradient of 0–0.5 M KCl in buffer A. PAGE analysis of the purified recombinant enzyme showed a single band in the presence of SDS.

RESULTS

Isolation and Characterization of Human cDNAs Encoding Deoxyhypusine Synthase—In the initial screening of the HeLa cDNA library with rabbit polyclonal antibodies against rat deoxyhypusine synthase, one positive clone, hDS-1a, was identified (Fig. 1). The deduced amino acid sequence of hDS-1a showed regions of identity to four tryptic peptides derived from the rat enzyme (Fig. 2, underlined sequences), but no sequence matching a fifth peptide (NGADYAVYINTAQe) was present. Of 25 positive clones isolated upon rescreening the library using a PCR-generated fragment of the hDS-1a insert as a
Construction of full-length and altered deoxyhypusine synthase cDNAs by PCR and the activities of the recombinant proteins

Each subclone was constructed from PCR amplification products using the template cDNAs and the sets of primers listed; the expression of recombinant proteins and determination of their activities were as described under "Experimental Procedures." In the case of 5'-terminal deletions, methionine was artificially introduced as the N\textsubscript{2}-terminal amino acid (hDS \textit{m4} and hDS \textit{m5}-encoded proteins). In the schematic representation, human coding sequences are black boxes, the deleted regions are white boxes, and the inserted yeast deoxyhypusine coding sequences are depicted by hatched boxes. The numbers indicate the amino acid residue number in the human coding sequence. A schematic diagram of the location of the primers in relation to the coding sequence of hDS-13 (Fig. 1) is shown below the table. n.d., not determined; m, mutant.

| Recombinant subclone or PCR product | Template | Primers | Coding region | Activity |
|------------------------------------|----------|---------|---------------|----------|
| 1. hDS (full-length clone)         | hDS-10 cDNA | Pr 5–1, Pr 3–1 | 1-369 | ++ ++ |
| 2. hDS \textit{m1} (5' & internal deletions) | hDS-1a cDNA | Pr 5–5, Pr 3–1 | 112-369 | n.d. |
| 3. hDS \textit{m2} (3' deletion) | hDS-10 cDNA | Pr 5–1, Pr 3–6 | 1-118 | n.d. |
| 4. hDS \textit{m3} (internal deletion) + hDS \textit{m2} cDNA | hDS-12 cDNA | Pr 5–1, Pr 3–1 | 1-269-318-369 | - |
| 5. hDS \textit{m4} (5' deletion) | hDS-10 cDNA | Pr 5–3, Pr 3–1 | 49-369 | - |
| 6. hDS \textit{m5} (5' deletion) | hDS-10 cDNA | Pr 5–4, Pr 3–1 | 98-369 | - |
| 7. hDS \textit{m6} (3' deletion) | hDS-10 cDNA | Pr 5–1, Pr 3–2 | 1-330 | - |
| 8. hDS \textit{m7} (5' deletion & insertion) | hDS-10 cDNA | Pr 5–6, Pr 3–1 | 194-369 | n.d. |
| 9. hDS \textit{m8} (3' deletion & insertion) | hDS-10 cDNA | Pr 5–1, Pr 3–4 | 1-193 | n.d. |
| 10. hDS \textit{m9} (insertion) + hDS \textit{m1} cDNA | hDS-12 cDNA | Pr 5–1, Pr 3–1 | 1-193-194-369 | ++ |

Location of primers

| nt 37 | Pr 3–6 | Pr 3–4 | Pr 3–2 | Pr 3–1 |
| ATG | Pr 5–3 | Pr 5–5 | Pr 5–6 | TGA |
| 1141 | Pr 3–3 | Pr 3–5 | 1141 |
hybridization probe, three independent clones (hDS-10, hDS-13, and hDS-20) were found to contain complete and identical open reading frames predicted to encode a protein of 369 amino acid residues. The nucleotide sequence and the deduced amino acid sequence of one such representative clone, hDS-13, are shown in Fig. 1. Upon comparison of the sequence of hDS-13 with that of hDS-1a, it is evident that, in hDS-1a, there is an internal deletion of 168 base pairs corresponding to a deletion of 56 amino acids (Asp262–Ser317) near the COOH terminus, in addition to a truncation of the 5'-terminal region (166 base pairs, 43 NH2-terminal amino acids). Except for the deletions underlined in Fig. 1, the sequences of the two clones are identical. To see if any other clone might have undergone the same internal deletion, PCR was performed using other positive clones as templates and the two primers, Pr5-5 and Pr3-3 (Table I), that flank the region of interest. The PCR results, taken together with analysis of the nucleotide sequences of this region in other clones, indicate that this deletion is unique for clone hDS-1a.

A comparison of the primary structure of human deoxyhypusine synthase with those of other species is shown in Fig. 2. The deduced amino acid sequence of the human enzyme is 58% identical and 73% similar to that of the yeast enzyme (21). Three regions of high sequence identity exist, encompassing residues 98–188, 201–248, and 277–330 of the human enzyme. In addition, the amino acid sequences determined for several tryptic peptides from the rat testis enzyme (20) or the Neurospora crassa enzyme (25) also are highly similar to the corresponding regions of the human and yeast enzymes. It is interesting to note that the human enzyme is shorter than the yeast enzyme. No counterpart sequence for 16 amino acids (Gln197–Asn212) of the yeast enzyme is present in the human enzyme.

Northern Blot Analysis—As shown in Fig. 3, one major transcript of ~1.4 kilobases was detected in RNA isolated from HeLa cells, human trophoblast cells (Fig. 3A), normal human epidermal keratinocytes (NHEK), or cell lines derived from head and neck tumors and dysplasias (Fig. 3B). Although deoxyhypusine synthase mRNA was detected in all the cell lines examined, its level appeared to be elevated in HeLa cells and in some of the tumor cell lines.

Purification of Human Recombinant Deoxyhypusine Synthase—Human deoxyhypusine synthase was expressed in E. coli BL21(DE3) cells in a time-dependent manner for up to 4 h after isopropyl-1-thio-D-galactopyranoside addition (data not shown). The enzyme activity was purified by a simple four-step protocol, summarized in Table III. After chromatography on Mono Q HR 10/10 (Fig. 4A), one major protein band, at ~41 kDa, was detected upon SDS-PAGE of the peak fractions (Fig. 4B). The specific activity of human recombinant deoxyhypusine synthase (0.9 × 10^6 units/mg of protein) was similar to that of the yeast recombinant enzyme (21) and of the enzyme purified from rat testis (20). Enzymatic Properties—The catalysis of deoxyhypusine formation in the eIF-5A precursor, from spermidine in the presence of NAD, by human recombinant protein defines it as deoxyhypusine synthase. Like the rat (7, 20) and yeast (21) enzymes, the human recombinant enzyme also catalyzes the cleavage of spermidine to diaminopropane and D1-pyrroline in the absence of the eIF-5A precursor (data not shown). The strict requirement of the human enzyme for NAD is also characteristic of the yeast enzyme. No counterpart sequence for 16 amino acids (Gln197–Asn212) of the yeast enzyme is present in the human enzyme.
characteristic of the rat and yeast enzymes. As with these enzymes, other nucleotides, including NADH, NADPH, FAD, and FMN, did not substitute for NAD with human deoxyhypusine synthase. Interestingly, the $K_m$ values for NAD (Table IV) are strikingly different for human, rat, and yeast enzymes, varying as much as 150-fold, whereas the $K_m$ values for spermidine and ece-$F$-5A are not very different (Table IV). The $K_m$ value for NAD for the human recombinant enzyme was 4.8 $\mu$M, 8-fold lower than that for the rat enzyme and 150-fold lower than that for the yeast recombinant deoxyhypusine synthase.

Deletion and Insertion Mutations—Since the initial clone, hDS-1a, had an internal deletion corresponding to the loss of 56 amino acids (amino acids 262–317), we designed a mutant clone, hDS$m^3$, with this deletion but with intact 5' and 3'-terminal coding sequences (Table II) in order to assess the importance of this region. The lysate of E. coli cells overexpressing protein from this clone was found to display no enzyme activity (Table II). Lysates of E. coli cells overexpressing recombinant proteins with truncation of either 48 or 97 amino acids from the NH$_2$ terminus or of 39 amino acids from the COOH terminus also exhibited no activity (Table II). In an effort to determine the basis of the drastic difference in the $K_m$ value for NAD for the human and yeast enzymes, we generated a mutant subclone, hDS$m^9$, with the partial nucleotide sequence corresponding to Gln197–Asn212 of the yeast deoxyhypusine synthase inserted into the human cDNA (Table II).

After expression of this clone, the resultant chimeric protein, isolated by ion-exchange chromatography on MonoQ HR10/10 (data not shown), displayed a specific activity ($6 \times 10^4$ units/mg of protein) significantly lower than that of the wild-type enzyme. Interestingly, the $K_m$ value determined for NAD ($7 \mu$M) for this aberrant enzyme was similar to the value for the wild-type human enzyme, rather than that for the yeast enzyme.

DISCUSSION

Hypusine is ubiquitous in all eukaryotic species, and its biosynthetic pathway appears to be highly conserved. This study shows that the human enzyme shares similar properties with deoxyhypusine synthases from diverse species, e.g. rat (7,
tween Thr98 and Ile330 of the human sequence. The known human and yeast deoxyhypusine synthase molecules, primary structure is highly conserved in the major portion of catalysis of a complex post-translational modification reaction.

3. Ammonium sulfate precipitation 144 21.1 146,000 65
2. Q-Sepharose 280 25.5 91,000 78
1. Cell lysate supernatant 540 32.6 61,000 100

TABLE III
Purification of recombinant human deoxyhypusine synthase

| Step                        | Protein | Total activity | Specific activity | Recovery |
|-----------------------------|---------|----------------|-------------------|----------|
|                             | mg      | 10^{-6} units/mg | %                 |          |
| 1. Cell lysate supernatant  | 540     | 32.6           | 61,000            | 100      |
| 2. Q-Sepharose              | 280     | 25.5           | 91,000            | 78       |
| 3. Ammonium sulfate precipitation | 144   | 21.1           | 146,000           | 65       |
| 4. Mono Q HR 10/10         | 15      | 13.5           | 900,000           | 41       |

20), N. crassa (25), and Saccharomyces cerevisiae (21), in the catalysis of a complex post-translational modification reaction. Primary structure is highly conserved in the major portion of human and yeast deoxyhypusine synthase molecules, i.e. between Thr98 and Ile330 of the human sequence. The known sequences of peptides from the rat (20) and Neurospora (25) enzymes also show a marked similarity to the primary structures of the human and yeast enzymes in this region. The maintenance of protein structure in these enzymes may have been mandated for the recognition of their three substrates, spermidine, NAD, and especially the highly conserved eIF-5A precursor, and, in turn, may have served to preserve a vital cellular function of eIF-5A. Despite the strict specificity of deoxyhypusine synthases from all species for this single cellular protein, cross-species reactivities between the enzymes and the eIF-5A precursor proteins are observed. As previously shown (21) for the yeast and rat enzymes, human deoxyhypusine synthase also catalyzes the modification of heterologous protein substrates, namely eIF-5A precursors from Chinese hamster ovary cells and yeast (data not shown).

FIG. 3. Northern blot analysis. Total RNAs (~25 µg) isolated from HeLa or human trophoblast cells (A) and normal human keratinocytes or tumor cell lines derived from head and neck cancers or dysplasias (B) were subjected to electrophoresis on a 0.66 M formaldehyde, 1% agarose gel and transferred to a nitrocellulose membrane by the method of Sambrook et al. (27). Both prehybridization and hybridization were conducted at 42 °C in 6 × SSC containing 50% formamide, 5 × Denhardt’s solution, 100 µg/ml yeast tRNA, and 0.1% SDS. After overnight hybridization with a cDNA probe, which was generated by PCR of hDS-1a cDNA using primers Pr5-2 and Pr3-5 and labeled with 32P, the membranes were washed at 23 °C for 10–20 min three times with 2 × SSC containing 0.1% SDS and once with 1 × SSC containing 0.1% SDS and then washed at 55–60 °C for 20 min three times with 0.2 × SSC containing 0.1% SDS. In A, 144 JAR is a human trophoblast cell line. In B, T 45 (28), HN 30 (29), HN 22 (29), HN 8 (29), and SCC 25 (30) are derived from oral squamous cell carcinomas; DOK (31) from premalignant keratinocytes; and NHEK (Clonetics Corp.) from normal human epidermal keratinocytes (source or reference given in parentheses). Kb, kilobases.

FIG. 4. Ion-exchange chromatography of human recombinant deoxyhypusine synthase on Mono Q. Protein (144 mg) from step 3 of Table III was applied to a Mono Q HR 10/10 column as described under “Experimental Procedures.” After washing the column for 10 min with buffer A, a salt gradient of 0–0.6 M KCl in buffer A was applied, and fractions of 2 ml (1 min) were collected. A, absorbance at 280 nm, conductivity, and enzymatic activity; B, the Coomassie Blue-stained pattern of an SDS-polyacrylamide gel (10%). Lane 1, crude E. coli lysate; lane 2, starting material before Mono Q chromatography; lane 3, fraction 41 (2 µl); lane 4, fraction 43 (2 µl); lane 5, standard marker proteins. All samples were heated in β-mercaptoethanol-containing SDS sample buffer prior to electrophoresis. The position of the human recombinant deoxyhypusine synthase protein is indicated by an arrowhead.

TABLE IV
Comparison of Km values for deoxyhypusine synthases from human, rat, and yeast

In the case of the human and yeast enzymes, highly purified recombinant enzyme from the respective cDNA expressed in E. coli was used. The rat enzyme was purified from testis (20). Human eIF-5A precursor protein (ec-eIF-5A) was used as the substrate for all determinations. Previously published values are given for the rat (7, 17, 20) and yeast (21) enzymes. Km values for the human enzyme were determined in a similar manner.

| Substrate | Human DS | Rat DS | S. cerevisiae DS |
|-----------|----------|--------|-----------------|
| Spermidine | 7.2 µM   | 4.5 µM | 6.1 µM |
| NAD       | 4.8 µM   | 40 µM  | 720 µM |
| ec-eIF-5A | 0.6 µM   | 0.4 µM | 0.9 µM |

* DS, deoxyhypusine synthase.

It is intriguing that the initial clone, hDS-1a, isolated by means of immunological screening, has an internal deletion that results in the loss of 56 amino acids (Asp262–Ser317). It was unclear whether this in-frame deletion reflects natural cellular processing of the deoxyhypusine synthase gene transcript or whether it arose as an artifact of expression library construction. Since no other clone with the same deletion was detected,
we sought to determine the existence of a natural mRNA(s) with this deletion. Using different sets of primers flanking the deletion region, PCR amplification of a HeLa cDNA mixture (HeLa QuickClone cDNA (CLONTECH) or cDNA prepared by a reverse transcriptase reaction of total RNA from HeLa cells) as a template thus far has provided no clear indication of the existence of a natural transcript with the corresponding deletion (data not shown). Determination of the genomic structure of deoxyhypusynase synthase might offer insight as to whether such a transcript can be generated by alternative splicing.

A mutant protein expressed from hDS m3, lacking Asp<sup>262</sup>-Ser<sup>317</sup> (corresponding to the internal coding region missing in hDS-1a), displayed no detectable deoxyhypusynase synthase activity. This lack of activity is not surprising since the deletion removed a highly conserved region of the protein (Fig. 2) and strongly indicates that this region is critical for enzyme activity. The mutant proteins expressed from clones hDS m4, hDS m5, and hDS m6 (Table II), with truncations of 48 or 97 NH<sub>2</sub>-terminal amino acids (Met<sup>1</sup>-Ala<sup>48</sup> or Met<sup>1</sup>-Cys<sup>97</sup>) or of 39 COOH-terminal amino acids (Asp<sup>333</sup>-Asp<sup>369</sup>), respectively, were also devoid of enzymatic activity, suggesting that, although not highly conserved, these regions also contain amino acid residues important for the proper conformation of the enzyme, necessary for binding substrates and/or for catalysis.

The most interesting difference between the primary structures of the human and yeast enzymes is the absence of a human sequence matching Gln<sup>197</sup>-Asn<sup>212</sup> in the yeast enzyme. Although this gap (indicated by . . . in Fig. 2) is located in the middle of a highly conserved region, the human recombinant deoxyhypusynase synthase exhibits as high a specific activity as the yeast enzyme, although some details concerning the steps in the biosynthesis of deoxyhypusine have been revealed (1). Little is known about the precise mechanism of the reaction. The availability of a human cDNA clone and its encoded protein should enable us to determine structural features of the active site and of the substrate binding domains as well as the residues involved in catalysis. A better understanding of the enzyme structure and the reaction mechanism will facilitate the development of potent and specific inhibitors of the human enzyme and pave the way to improved means of cellular regulation through the inhibition of hypusine biosynthesis.

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