N-terminal Extension of Canine Glutamine Synthetase Created by Splicing Alters Its Enzymatic Property*

Daesung Shin and Chankyu Park‡

From the National Creative Research Initiative Center for Behavioral Genetics, Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejon 305-701, Republic of Korea

It was found that an extra exon exists in the first intron of glutamine synthetase gene, generated by means of alternative splicing (Shin, D., Park, S., and Park, C. (2003) Biochem. J. 374, 175–184). Inclusion of this exon decreased the translation of glutamine synthetase (GS) in human, dog, and mouse. When translated in vitro with the canine GS transcript containing the exon, we obtained two different species of GS enzymes. Besides the known 45-kDa protein, the extended form of GS was identified with additional 40 amino acids on its N-terminal end. An upstream ATG in the extra exon served as a translation initiator for the long form of GS. When the long transcript was translated in vivo in animal cells, only the long GS was expressed. On the other hand, the long GS is less predominant relative to the short one in different tissues including brain and liver. Subcellular fractionation of canine brain revealed that the long GS is present in all cellular compartments as is the short one, which is consistent with fluorescence microscopy data obtained with green fluorescent protein fused to GS. The short (SGS) and long (LGS) forms of canine GS were purified in Escherichia coli and shown to have similar \( K_m \) values for L-glutamate and hydroxylamine. However, the \( K_m \) values for ATP were slightly altered, 1.3 and 1.9 mm for the short and long GSs, respectively. The \( K_m \)S for L-methionine-S-sulfoximine (MSOX), a highly potent ATP-dependent inactivator of GS, were considerably different such that the values are 0.067 and 0.12 mm for the short and long GSs, respectively. When the intrinsic fluorosciences of tryptophans were monitored upon bindings of chloride and metal ions without any effect on the oligomeric state, the pattern of quenching in LGS was significantly different from that of SGS. Taken together, the N-terminal extension in the long isoform of GS induces a conformational change of core enzyme, leading to a change in affinity to its substrates as well as in the effector-induced conformational alterations.

Glutamine synthetase (GS\( ^\ddagger \); EC 6.3.1.2; L-glutamate ammonia ligase) is an enzyme that catalyzes the ATP-dependent conversion of glutamate and ammonia into glutamine, and thus plays a critical role in eliminating the excitotoxid glutamate in animal brains (1). The expression of eukaryotic GS is regulated at transcriptional and post-transcriptional levels and becomes unstable with oxygen mediated by metal or free radical peptides, generated from a fragmentation of \( \beta \)-amyloid, and by growth hormone or glutamine (2).

The mammalian GS protein has been reported to form an eight-subunit oligomer (3) with unknown three-dimensional structure. The bindings of chloride and manganese/magnesium ions to allosteric and activator sites of GS, respectively, cause changes in GS conformation, without affecting its oligomerization (4). As an essential trace metal in vivo, the manganese ion is mostly (\( \sim 80\% \)) bound to GS in astrocytes (5), although its concentration is variable in other mammalian tissues. Magnesium is also bound to mammalian GS in vivo (6). L-methionine-S-sulfoximine (MSOX), a structural analogue of L-glutamate, is converted to MSOX-phosphate, mimicking the tetrahedral intermediate formed between an enzyme-bound \( \gamma \)-glutamyl phospho- and ammonia at the active site of GS (7). This reaction product becomes an irreversible and noncovalent inhibitor of the enzyme. MSOX was originally isolated from nitrogen chloride-treated zein and characterized as a toxin causing induction of convulsions, hysteria, and epileptic fits in a number of animals (8). The \( K \) values of MSOX are 105, 1, 161, and 100 \( \mu \)M for GS proteins of sheep, Escherichia coli, pea, and spinach, respectively (8). Despite its crucial role in many neurological diseases (9–13), information on mammalian GS is rather scarce, and inconsistencies are found in various reports on its mass and subunit arrangements (8).

In vertebrate, compartment-specific GS isozymes are produced from a single gene and targeted to either mitochondria or cytosol in a tissue-specific manner (14). The N terminus of GS is variable in size and sequence and serves as a subcellular targeting signal (15). Besides the known 44-kDa protein, the GS-like protein of 54 kDa was reported from human brain. This enzyme is mostly found in crude mitochondrial fractions and possesses higher hydroxylamine-L-glutamine transferase activity than the 44-kDa GS (16, 17).

Modulation of the translational efficiency of mammalian GSs by 5’ transcript leader region has been suggested as a mechanism for regulating its expression (18). This phenomenon was studied in detail with the canine GS, a 45-kDa enzyme with 373 amino acids, which is translated from the gene of \( \sim 10 \) kb in length, being organized into seven exons and six introns. An extra exon is found in the first intron of glutamine synthetase gene, which is subjected to an alternative splicing. The long transcript with extra 5’-UTR is translated less efficiently than the short one. This translational regulation is partially due to an abortive initiation at the upstream ATG located in the extra exon (18). It is known that the 5’ transcript leader regions in most vertebrate mRNAs are less than 100 nucleotides long.

This paper is available on line at http://www.jbc.org
from the cells, and the induction medium containing Dulbecco’s plus reagent (Invitrogen) according to the manufacturer-supplied protocol. The GS protein was characterized to have different enzymatic properties, presumably due to a conformational alteration.

**EXPERIMENTAL PROCEDURES**

**Reagents and Primers**—All reagents used in the present study were purchased from Sigma, unless otherwise stated. PCR primers for subcloning were synthesized by Genotech co. (Taejon, Korea). PCR products were separated by agarose gel electrophoresis, purified using QIAquick Gel Extraction kits (Qiagen). All plasmid constructs were sequenced using the Big Dye termination kits for the ABI Prism 3100 DNA sequencer (PerkinElmer Life Sciences).

**Animal Tissues**—Tissues from the frontal lobe, heart, skeletal muscle, kidney, and liver of dog (Sapsari, a Korean breed, 22) were obtained from an adult animal, with an approval by the University Animal Care and Use Committee at KAIST (Taejon, Korea). The brain was obtained from an adult animal of the FVB strain, which was purchased from Daehan Breeding Center Co. Ltd (Seoul, Korea) and housed under controlled temperature and lighting (22 °C with 12-hour light-dark cycle) with free access to food and water, according to the National Institutes of Health guideline for the care and use of laboratory animals. The brain tissue of human was a mixture of samples from the parietal lobe, cortex, and white matter, kindly provided by Chong-Jai Kim (Seoul National University, College of Medicine, Seoul, Korea), with an inferred consent of 16-year-old male who died of primitive neuroectodermal tumor in the chest wall and an approval by the Ethical Committee of Seoul National University Hospital (Seoul, Korea).

**In Vitro Transcription and Translation**—The short and long transcripts of canine GS, covering the exon 1 to exon 7, were reverse transcribed with oligo(dt)20, amplified with primers cGSBamHI-f (5′-GGGGATCCTAGGCTGACAGGAACTGGACGGG-3′), AF544242, nt 1–20) and cGSXhoI-r1 (5′-GGCGTCGAGTTTGTATTCGAGAAGGGG-3′, AF544242, nt 1369–1349), and subcloned into the BamHI and XhoI sites of pcDNA3.1(+ (Invitrogen), yielding pSGS and pLGS, respectively. The linearized templates of 5μg by digestion with XhoI were used for in vitro transcription using the mMESSAGEmMACHINE™ kit (Ambion) to obtain capped transcripts. The integrity of transcripts was ascertained by visualizing on denaturating gel.

**Purification of the Recombinant GS Enzymes**—The short and long coding sequences of canine GS were amplified with primers cGSNdel-f (5′-GGGATCCATGGCTGACAGGAACTGGACGGG-3′, AF544242, nt 1–20) and cGSXhoI-r1 (5′-GGCGTCGAGTTTGTATTCGAGAAGGGG-3′, AF544242, nt 1369–1349), and subcloned into the Ndel and XhoI sites of pET21b (Novagen) to obtain the pET-SGS and pET-LGS plasmids. E. coli strain BL21(DE3) was transformed with pET-SGS or pET-LGS to produce recombinant GS protein. The protein was induced with a treatment of 0.5 mM isopropyl-1-thio-galactopyranoside to cells of 0.3–0.4 O.D. at 600 nm. The cells were then allowed to grow 4 h more at 30 °C and harvested by centrifugation with JA14 rotor at 4 °C and 8000 rpm for 15 min. The pelleted cells were resuspended in 5 ml imidazole/0.5 M NaCl/20 mM Tris-Cl (pH 7.9), sonicated, and centrifuged 25,000 rpm in SW41Ti rotor for 30 min at 4 °C. The supernatant was filtered through a 0.45 μm filter, passed through a nickel-nitrilotriacetic acid agarose column (Qiagen) that had been equilibrated with the same buffer. The column was washed with 15 volumes of the previous buffer and 6 volumes of 50 μM imidazole/0.5 M NaCl/20 mM Tris-Cl (pH 7.9), collected with the fractions of 1 ml each, and pooled for the active fractions. Purity was assessed by 10% SDS-PAGE as described by Sambrook and Russell (25), and protein concentration was determined with a dye-binding assay (Bio-Rad) using bovine serum albumin as a standard. The purified protein was dialyzed against 10 mM imidazole-HCl/2 mM EDTA/5 mM 2-mercaptoethanol (pH 7.2) to store at 4 °C, as reported by Listrom et al. (26). Protein concentration was determined using the method of Lowry et al. (38) using bovine serum albumin as a standard.

**Western Blotting Analysis**—Protein samples were heat denatured in the presence of 2-mercaptoethanol and SDS and separated electrophoretically in a 10% SDS-polyacrylamide gel and visualized by autoradiography.

**Fluorescence Spectroscopy**—1 μg of GS protein in 10 mM HEPES-
**RESULTS**

**Novel Translation Initiation from the Alternative Transcript of Canine Glutamine Synthetase Gene**—It was found previously that alternatively spliced GS transcript acquiring extra 5'-UTR decreased the normal translation (18). In an effort to obtain further evidences on this observation, we carried out an in vitro translation of transcripts made from the GS plasmids in rabbit reticulocyte lysate. Although the short transcript generated a 45-kDa product, whereas the long transcript produced an additional 49-kDa band, each designated here as SGS and LGS, respectively. Both canine GS transcripts were cloned into the expression vector, yielding pSGS for short and pLGS for long ones. They were transfected into 293T cells and analyzed by Western blotting with monoclonal antibody for GS. In the negative control (Mock), no protein was detected (2). The plasmid harboring short transcript (pSGS) produces a 45 kDa product, as in the case of in vitro translation, whereas the pLGS produces only 49 kDa protein without the short form. When both plasmids were transfected with equal molarity, similar amounts of two proteins were detected (pSGS+LGS).

Because the rabbit reticulocyte, a highly specialized cell, is known to have ill-balanced translation system (28), expressions of the GSs were analyzed transiently in 293T cells (Fig. 1B). The 293T cells transfected with the plasmid containing cDNA for SGS (pSGS) produced a band of expected size (45 kDa) on Western blot, whereas the LGS plasmid (pLGS) generated only the 49-kDa protein band, which is different from the result of in vitro translation (Fig. 1A). When both pSGS and pLGS were transfected, equal amounts of proteins were expressed. The same patterns of GS expressions were obtained for cDNAs of SGS and LGS in SK-N-SH cells (data not shown), suggesting that the primary role of long GS transcript is to produce the LGS protein in vivo.

**The Long Form of GS Is Found in Canine Brain and Liver Tissues**—To examine the presence of LGS in the canine tissue, soluble extracts from the frontal lobe, heart, skeletal muscle, kidney, and liver tissues were separated on SDS-PAGE and blotted with monoclonal antibody raised against the mouse GS. The canine GSs are highly expressed in brain, moderately in kidney, and liver tissues were separated on SDS-PAGE and blotted with monoclonal antibody raised against the mouse GS. The canine GSs are highly expressed in brain, moderately in kidney, and liver tissues were separated on SDS-PAGE and blotted with monoclonal antibody raised against the mouse GS. The canine GSs are highly expressed in brain, moderately in kidney, and liver tissues were separated on SDS-PAGE and blotted with monoclonal antibody raised against the mouse GS.

![Image](60x364 to 561x501)

**FIG. 1. Translations of canine GS transcripts in vitro and in vivo.** A, the two canine GS transcripts synthesized in vitro were translated in a rabbit reticulocyte lysate, which were analyzed by SDS-PAGE and autoradiography. Unlike the short transcript (Short), the long one (Long) has extra 5'-UTR corresponding to exon 1. Equal amounts of capped transcripts (0.5/0.5 pmol; 1/1 pmol per 50 μl reaction) for short and long were used. Translation from the short transcript generated a 45-kDa product, whereas the long transcript produced an additional 49-kDa band, each designated here as SGS and LGS, respectively. B, both canine GS transcripts were cloned into the expression vector, yielding pSGS for short and pLGS for long ones. They were transfected into 293T cells and analyzed by Western blotting with monoclonal antibody for GS. In the negative control (Mock), no protein was detected (2). The plasmid harboring short transcript (pSGS) produces a 45 kDa product, as in the case of in vitro translation, whereas the pLGS produces only 49 kDa protein without the short form. When both plasmids were transfected with equal molarity, similar amounts of two proteins were detected (pSGS+LGS).

![Image](103x598 to 519x738)

**FIG. 2. The sequence of extra exon and its corresponding peptide.** The exon 1′ contained in the long transcript is shaded with its encoded amino acids shown below the codon sequences. The translation of SGS is initiated from the start codon (+1), whereas the one for LGS is located at −120.

KOH (pH 7.2) was mixed with KCl, MnSO4, or MgSO4 at the indicated final concentration and incubated at 25 °C for 10 min. Intrinsic fluorescence was monitored with the 8100 Series 2 spectrophotometer (SLM-AMINCO) equipped with a temperature-controlled cuvette compartment maintained at 25 °C. The tryptophan fluorescence being excited at 295 nm were monitored in the range of 310–400 nm.

**Analytical Gel Filtration Chromatography**—The GS protein was passed through 0.2-μm nylon filter (Whatman) and loaded onto a Superdex 200 column (bed dimensions, 10 × 300 mm; Amersham Biosciences) equilibrated with 10 mM HEPES-KOH (pH 7.2) at 25 °C. The protein peaks were detected with a AKTAPrime Chromatography system and PrimeView software (Amersham Biosciences) with an absorbance at 280 nm. The apparent molecular masses were estimated using intrapolation in the plots of Vr (elution volume) – Vo (void volume) versus log [molecular weight] from standard proteins (Amersham Biosciences) of chymotrypsinogen A (19.9 kDa), albumin (64.7 kDa), catalase (219 kDa), and thyroglobulin (689 kDa). The void volume (6.2 ml) was determined using blue dextran 2000 (1 mg/ml).

Because the rabbit reticulocyte, a highly specialized cell, is known to have ill-balanced translation system (28), expressions of the GSs were analyzed transiently in 293T cells (Fig. 1B). The 293T cells transfected with the plasmid containing cDNA for SGS (pSGS) produced a band of expected size (45 kDa) on Western blot, whereas the LGS plasmid (pLGS) generated only the 49-kDa protein band, which is different from the result of in vitro translation (Fig. 1A). When both pSGS and pLGS were transfected, equal amounts of proteins were expressed. The same patterns of GS expressions were obtained for cDNAs of SGS and LGS in SK-N-SH cells (data not shown), suggesting that the primary role of long GS transcript is to produce the LGS protein in vivo.
in 293T cells. The 293T cells transfected with the plasmid containing cDNA for the short transcript of human GS produced the band of expected size (45 kDa), so did the long one with less amount of protein (data not shown). The short transcript of mouse GS expresses the 45-kDa protein, whereas the long one produces none (not shown), presumably due to translational repression as described previously (18).

Intracellular localization of GS protein was analyzed by subcellular fractionation of the canine brain, which were blotted with GS antibody. Absolute amounts of both enzymes were varied in different fractions, i.e. cytosol (S3), endoplasmic reticulum/plasma membrane (P3), nuclei (P1), and other particulate fraction (P2). However, their ratios are about the same, suggesting that the extra 40 amino acids are not associated with specific targeting. This pattern of SGS/LGS distribution is consistent with the result of subcellular localization of the GS proteins fused to GFP, produced from pEGFP-SGS and pEGFP-LGS. When two constructs were transfected into Madin-Darby canine kidney cells, the GS-GFP fusion proteins were expressed evenly in all subcellular regions (Fig. 4B).

### The Glutamine Synthetase Isoforms Differ in Their Enzymatic Properties

The two different canine GS genes were cloned into an inducible pET21b vector, from which the enzymes were purified to homogeneity. The colorimetric synthetase reaction assay with L-glutamate, hydroxylamine, and ATP as substrates revealed that $K_m$ values of SGS and LGS are $1.1 \pm 0.12$ and $1.3 \pm 0.14$ mM for L-glutamate, $1.6 \pm 0.41$ and $1.7 \pm 0.31$ mM for hydroxylamine, and $1.3 \pm 0.24$ and $1.9 \pm 0.23$ mM for ATP, respectively (Table I). The values for L-glutamate and hydroxylamine are similar in both enzymes, but the affinity for ATP is higher in SGS than in LGS. It is known that MSOX, with the presence of ATP, is phosphorylated by GS, leading to an irreversible and non-covalent inhibition of the enzyme (27). When MSOX was titrated in the presence of 50 mM L-glutamate, the $K_i$ values were 0.067 and 0.124 mM for SGS and LGS, respectively (Table I), indicating that the affinity for MSOX is lower in LGS than in SGS. The affinity changes for ATP and MSOX suggest that the binding sites for both

---

**Fig. 3. Detection of GS proteins in mammalian tissues.** A, results of Western blotting analysis for five different canine tissues. Soluble extract of each 10 µg of tissue was separated by SDS-PAGE, transferred onto nitrocellulose membrane, and detected by GS monoclonal antibody. Canine GS protein is highly expressed in frontal lobe and moderately in liver. SGS is more abundant than LGS. B, equal amounts of mouse, human, and canine brain tissues were analyzed by SDS-PAGE and Western blot. LGS was detected only in canine brain tissue, not in mouse and human.

**Fig. 4. Intracellular localizations of the two glutamine synthetases.** A, subcellular fractions of canine brain were analyzed by Western blot. The SGS and LGS were detected highly in cytosol (S3) and endoplasmic reticulum/plasma membrane (P3), and moderately in nuclei (P1) and other particulate fraction (P2). B, the GFP protein was fused to the downstream of GS open reading frame and transiently expressed in Madin-Darby canine kidney cells (dog kidney). The pEGFP-SGS and pEGFP-LGS plasmids produce the GFP-fused SGS and LGS, respectively. The fluorescence patterns (left) and the matching phase-contrast images (right) are shown.

**Table I**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (μmol/min per nM)</th>
<th>SGS</th>
<th>LGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-glutamate</td>
<td>$1.1 \pm 0.12$</td>
<td>$0.26 \pm 0.0058$</td>
<td>$1.3 \pm 0.14$</td>
<td>$0.27 \pm 0.0064$</td>
</tr>
<tr>
<td>MSOX</td>
<td>$0.067 \pm 0.0030$</td>
<td>$0.124 \pm 0.0051$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>$1.3 \pm 0.25$</td>
<td>$0.32 \pm 0.0157$</td>
<td>$1.9 \pm 0.23$</td>
<td>$0.31 \pm 0.0090$</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>$1.6 \pm 0.41$</td>
<td>$0.31 \pm 0.0276$</td>
<td>$1.7 \pm 0.31$</td>
<td>$0.32 \pm 0.0259$</td>
</tr>
</tbody>
</table>
substrates are altered due to the presence of N-terminal 40 amino acids of LGS.

Fluorescence Detection of Conformational Differences in GSs upon Bindings of Various Effectors—Maurizi et al. (4) demonstrated that bindings of various effectors, such as chloride, manganese, and magnesium, to allosteric or activator site of the bovine brain GS cause a conformational change as monitored by fluorescence quenching, without affecting the oligomerization state. There are seven tryptophans and fifteen tyrosines in the short GS protein, whereas the long one contains an extra tryptophan in the N-terminal peptide (Fig. 2). To assess whether the extended peptide of LGS affects the conformation of GS, we monitored fluorescence quenching by the presence of KCl, MnSO₄, or MgSO₄, each treated alone or combined together. The fluorescence changes are shown here as the ratio of fluorescence (F) for the sample to that of SGS.
enzyme itself (Fig. 5, F.). When excited at 295 nm for tryptophan, addition of MnSO₄ to SGS resulted in fluorescence quenching of about 15%, whereas MgSO₄ increased fluorescence of 30% (Fig. 5). In contrast, LGS shows 34% quenching with MnSO₄, and only 8% increase with MgSO₄. Nevertheless, the patterns of fluorescence changes are similar. However, the tryptophan fluorescence of SGS increased −133% by an addition of 10 mU chloride, whereas no change was observed in LGS. Further addition of MnSO₄ and MgSO₄ to the chloride-treated SGS resulted in 85% quenching relative to the untreated. For LGS, quenching was 17% with MnSO₄, whereas MgSO₄ increased fluorescence of about 5–6%. The notable differences in fluorescence between SGS and LGS were observed upon chloride binding, with enormous increase in SGS. Based on the fact that chloride ion binds to the allosteric sites of GS, such fluorescence changes may reflect conformational differences between the two enzymes.

On the other hand, the basal levels of tryptophan fluorescences are different between SGS and LGS such that the emission of LGS at 338 nm is 1.5-fold higher than that of SGS. The difference could be due to either the presence of extra tryptophan residue in LGS or the change in microenvironment of other tryptophans, or both. To exclude the possibility that the LGS is caused by its oligomerization, we examined oligomeric states of the enzymes by gel-filtration chromatography. As shown in Fig. 6, the degrees of multimerization in both proteins are similar, in which the majority exists as octamer of 70–73%. This suggests that the fluorescence changes in the GS proteins are not associated with their quaternary structures.

**DISCUSSION**

We characterized two isoforms of GS proteins, 45 and 49 kDa, translated from one functional gene. The long form was generated by in-frame translation from the upstream ATG located in the extra exon of long GS transcript. The extra peptide did not reveal any sequence similarity in the data base search. Because the sequence analysis predicted O-linked glycosylation site for N-acetylglucosamine at +40 position (threonine) of LGS, we treated LGS with β-acetyl hexosaminidase or with sodium hydroxide for β-elimination (29). However, the result was negative (data not shown), although we cannot completely rule out the possibility of other modifications including phosphorylation.

In a previous study (18), it was shown that the ratio of the short to long transcripts of canine GS was similar in various brain tissues. However, the relative ratio of the proteins was different, with far more SGS (Fig. 3A), implying that there is a difference in stability or translation between the transcripts of LGS and SGS. The known regulators of GS expression at the transcriptional level include glutamine, growth hormone, and several other hormones and neurotransmitters, which affects functional activities or intracellular location of the GS protein.

**N-terminally Extended Glutamine Synthetase**

We compared enzymatic properties of the two enzymes, in which the affinities for L-glutamate and hydroxylamine are similar, although those for ATP and MSOX were different (Table I). This may indicate that there is a difference in enzyme conformation, at least in the substrate binding sites for ATP and MSOX. To further investigate this, we examined chloride and divalent metal ion bindings to allosteric and activator sites, respectively, which induce the conformational changes of GS (4). The most prominent differences in fluorescence changes were found in the chloride binding, in which a considerable increase was observed for the short protein compared with the long one, suggesting a conformational difference between the two proteins.

Because the fluorescence change could also be due to a nonspecific binding of chloride on the substrate site if there is a fluorophore located in the substrate site of GS, we examined the fluorescence changes upon bindings of ADP, L-glutamate, and MSOX. The marginal difference in fluorescence was detected below the level of 5%, indicating that there is no tryptophan residue in the substrate site that significantly alters its fluorescence upon substrate binding (data not shown). On the other hand, to assess whether the fluorescence change is due to the oligomerization of enzyme, we detected fluorescence in the sample with only monomers, obtained by treating 1% CHAPS (witterionic detergent). Here, the fluorescence change was less than 10% of the values obtained for purified protein with mostly oligomeric forms, indicating that the tryptophan fluorescence is not probing the oligomerization (data not shown).

We attempted to detect any structural difference due to an extension of N-terminal amino acids in LGS using circular dichroism spectroscopy. However, an analyzable spectra were not obtained because of the high propensity of GS oligomerization that disturbs circular dichroism measurement. At any rate, we already presented other enzymatic and spectroscopic data indicating the conformational alteration by an extension of N-terminal amino acids in LGS, although physiological
relevance of this structural variation still remains to be elucidated.

REFERENCES