J. Microbiol. Biotechnol. (1998), **8**(6), 663 – 668

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

Polyglutamine Residues from Machado-Joseph Disease Gene Enhance Formation of Aggregates of GST-Polyglutamine Fusion Protein in *E. coli*

RHIM, HYANGSHUK[1], KYOUNG-SOOK BOK, MI-JEONG CHANG[1], IN-KYUNG KIM[1], SUNG SUP PARK[2], AND SEONGMAN KANG*

Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea
[1]Research Institute of Molecular Genetics, College of Medicine, Catholic University of Korea, Seoul 137-701, Korea
[2]College of Medicine, Seoul National University, Seoul 110-744, Korea

Received: September 7, 1998

Abstract Several neurodegenerative diseases such as Huntington's disease, dentatorubralpallidoluysian atrophy, spinobulbar muscular atrophy, Machado-Joseph disease, and spinocerebellar ataxias type 1 are associated with the aggregation of expanded glutamine repeats within their proteins. Generally, in clinically affected individuals, the expansion of the polyglutamine sequences is beyond 40 residues. To address the length of polyglutamine that forms aggregation, we have constructed plasmids encoding glutathione S-transferase (GST) Machado-Joseph disease gene fusion proteins containing polyglutamine and investigated the formation of aggregates in *E. coli*. Surprisingly, even $(Gln)_8$ in the normal range as well as $(Gln)_{65}$ in the pathogenic range enhanced the formation of insoluble protein aggregates, whereas $(Ser)_8$ and $(Ala)_8$ did not form aggregates. Our results indicate that the formation of protein aggregates in GST-polyglutamine proteins is specifically mediated by the polyglutamine repeat sequence within their protein structure. Our study may contribute to the understanding of the molecular mechanism of the formation of protein aggregates in neurodegenerative disorders and the development of preventative strategies.

Key words: Polyglutamine, aggregates, MJD gene, GST-fusion proteins

CAG expansion diseases include Machado-Joseph disease (MJD), Kennedy's disease, spinocerebellar ataxia type I (SCA1), Huntington's disease (HD), and dentatorubral-pallidoluysian atrophy (DRPLA) [7, 15, 21]. These diseases contain an expanded tract of glutamines within their proteins. Whereas a normal repeat length (7~37 repeats) has no pathological consequence, expansion of the glutamine tract beyond 40 repeats leads to neuronal loss and a degenerative phenotype: the longer the repeat, the earlier the onset and the more severe the disease. The CAG expansion is associated with anticipation whereby the penetrance of the disease is increased in successive generations.

Although there are many similar features, the disorders differ in many respects. Symptoms differ noticeably, ranging from progressive motor weakness in Kennedy's disease to cognition failure and chorea in HD [7, 18, 21]. An important feature of CAG expansion diseases is the differing neuronal selectivity among the various diseases. For instance, the primary affected site in Kennedy's disease is motor neurons and that in HD is the neurons of the striatum. The selective patterns of neurodegeneration occur despite the fact that the disease proteins are widely expressed in both brain and peripheral tissues [14, 21]. Several factors may contribute to the neuronal selectivity, including specific interactions with other proteins whose expression is spatially or temporally restricted and the particular protein context within which the glutamine resides [12]. The proteins that are known to interact with huntingtin (HD protein) include glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an unidentified calmodulin-associated protein, huntingtin-associated protein (HAP1), and a protein homologous to the yeast cytoskeleton-associated protein Sla2p (HIP1) [2, 3, 10, 12]. Ataxin-1, the gene defective in SCA1, interacts with a GAPDH protein fragment that contains the NAD1-binding domain and the first 21 residues of the catalytic domain. Kahlem *et al.* [9] demonstrated that polyglutamine domains are good substrates of transglutaminase (TGase) and that, generally, the longer the polyglutamine domain, the greater the activity.

Recent evidence shows that expanded polyglutamine itself may drive the degenerative process. Isolated, expanded glutamine tracts cause programmed cell death in transfected cells and progressive neurodegeneration in transgenic

*Corresponding author
Phone: 82-2-3290-3448; Fax: 82-2-927-9028;
E-mail: skang@kuccnx.korea.ac.kr

mice. Ordway *et al.* [13] showed that an expanded glutamine repeat inserted into a non-disease protein causes neurodegeneration in transgenic mice. Recent studies *in vitro* reveal that an expanded polyglutamine protein forms insoluble amyloid-like fibrils [4, 13]. Analysis of neuronal nuclear inclusions (NI) in transgenic animals and in HD brains reveals occasional fibrils within the NI, consistent with amyloid-like deposition. Although it is unknown whether NI cause the disease or simply reflect the disease process, the fact that they are preferentially found in susceptible neurons indicates that they are intimately linked to disease progression. These results suggest that the aggregation of expanded polyglutamine proteins underlies neurodegeneration in these diseases.

To address the length of polyglutamine that forms aggregations, we have constructed plasmids encoding glutathione S-transferase (GST) Machado-Joseph disease gene fusion proteins and investigated the formation of aggregates in *E. coli*. MJD1 is the gene defective in SCA3/MJD, the most common dominantly inherited ataxia worldwide [8, 14]. The glutamine repeat, which is located near the carboxyl terminus of the protein, normally contains 13~36 repeats and becomes expanded to 100 repeats in disease. Here, we show that even $(Gln)_8$ in the normal range as well as $(Gln)_{65}$ in the pathogenic range induce the formation of insoluble protein aggregates in *E. coli*, whereas $(Ser)_8$ and $(Ala)_8$ did not induce aggregates. This paper may contribute to the understanding of the molecular mechanism of the formation of protein aggregates in neurodegenerative disorders and the development of preventative strategies.

MATERIALS AND METHODS

Construction of Expression Plasmids for GST-Fusion Proteins Containing Polyglutamine Residues

For the construction of plasmids used to examine *in vitro* aggregation of proteins containing polyglutamine residues, genomic DNAs from a normal individual and a Korean Machado-Joseph disease (MJD) patient were used as the starting template to amplify the fragment containing CAG repeats by polymerase chain reaction (PCR). PCR reaction mixtures (50 μl) contained 50 ng of genomic DNA as a template, 50 pmols of F-MJD (5') and R-MJD (3') as primers, 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 100 μg/ml nuclease-free BSA, and 2.5 units of cloned *Pfu* DNA polymerase (Stratagene). The sequences of F-MJD and R-MJD were GCG **CCT CGA GGG ATC C**GC CAT GAT ATA CTT CAC TTT TGA A (40 mer; *Xho*I and *Bam*HI restriction enzyme sites: bold) and GC**G CGG CCG CGA ATT C**TT ATG TCA GAT AAA GTG TGA AGG T (40 mer; *Not*I and *Eco*RI restriction enzyme sites: bold), respectively. The thermocycling conditions used were 94°C for 1 min, 40°C for 1 min, and 72°C for 3 min for 20 cycles followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 30 cycles. The amplified fragments containing CAG repeats in the normal $(CAG)_8$ and expanded $(CAG)_{65}$ that contained *Bam*HI (5') and *Eco*RI (3') sites were inserted into pGEX-2T [1] that was digested with *Bam*HI and *Eco*RI. The resultant plasmids were named pGST-$(Gln)_8$ and pGST-$(Gln)_{65}$. The plasmids constructed in this study were sequenced by dideoxy sequencing to verify their identity.

Construction of Frameshift Mutants

For the construction of the GST-$(Ala)_8$ and GST-$(Ser)_8$, polymerase chain reactions were performed using oligomers MJD-5 (5') and R-MJD (3') as primers and pGST-$(Gln)_8$ and pGST-$(Gln)_{65}$ as the template to create +1 (polyserine residues) and +2 (polyalanine residues) frameshift mutants of polyglutamine residues (Fig. 1). The sequence of MJD-5 is GCG **CAG ATC TCG GAT CC**G AAT GTT TCA GAC (30 mer; *Bgl*II and *Bam*HI restriction enzyme sites: bold). These PCR amplified frameshift fragments were inserted into the *Bgl*II and *Bam*HI-digested pGEX-2T vector. The constructed plasmids were sequenced by dideoxy sequencing to verify their identity. Details of plasmids are available upon request.

Construction of Expression Plasmids for the Other GST-Fusion Proteins

pGST-synuclein was constructed by inserting the full length of α-synuclein (the coding region of 1 to 140 amino acid residues: Genebank accession number L08850), which was amplified by the PCR of cDNA isolated from lymphocytes of a normal person, into pGST-PL, a pGEX-2T derivative, which contains the multiple cloning sites originated from pEGFP-N1 (Clontech). pGST-NAC was

ata tac ttc act ttt gaa tgt ttc aga **[cag cag caa aag cag caa (cag)$_n$ cgg gac cta tca**
1 Y F T F E C F R Q Q Q K Q Q (Q)$_n$ R D L S
N V S D S S K S S N (S)$_n$ G T Y Q
M F Q T A A K A A T (A)$_n$ G D I
gga cag agt tca cat cca tgt gaa agg cca gcc acc agt tca gga gca ctt ggg agt gat
G Q S S H P C E R P A T S S G A L G S D
D R V H I H V K G Q P P V Q E H L G V I
R T E F T S M *
cta ggt aag gcc tgc tca cca ttc atc atg ttc gct acc ttc aca ctt tat ctg aca taa]
L G K A C S P F I M F A T F T L Y L T *
*

Fig. 1. Sequence of a genomic segment of SCA3/MJD containing a CAG repeat used in this study.

Shown in the sequence is an exon (bold) and an intron (italicized) of a segment of MJD genes that were joined to the carboxyl terminus of glutathione-S-transferase (GST). Genomic DNAs from a normal individual and a MJD patient contain 8 repeats of CAG and 65 repeats of CAG in the MJD region, respectively. Their identity was verified by dideoxy sequencing. The amino acid sequences that are encoded by the sequences are shown: top, middle, and bottom lines contain polyglutamine, polyserine, and polyalanine residues, respectively. The asterisks represent termination codons.

constructed by the PCR ampification of 35 residues (amino acid residues 61 to 95) of α-synuclein followed by inserting into pGST-PL. pGST-anti-Xpress was created by PCR amplification of the multiple cloning site for pcDNA3.1/HisC (Invitrogen) followed by inserting into pGST-PL. pGST-Tat-1(86R) and pGST-Tat-2(130R) were described previously [17].

Expression and Purification of GST-Fusion Proteins Using Ionic Detergent

The GST-fusion proteins were expressed in *E. coli* strain XA 90 [5]. For routine preparations of GST-fusion proteins, 2.5 ml cultures were grown overnight at 37°C in Luria Broth (LB) medium containing 50 μg/ml ampicillin [1, 6, 16]. After overnight culture, the stationary phase cultures of *E. coli* harboring GST-plasmids were diluted 1:10 into 25 ml of fresh LB containing ampicillin and grown for another 1.5 h at 37°C. GST-fusion proteins under control of the Ptac promoter were then induced for another 3.5 h with a final concentration of 0.5 mM IPTG (isopropyl-1-thio-β-D-galactoside, Sigma). Cells were harvested and suspended in 50 μl of 1×SDS loading buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 144 mM 2-mercaptoethanol, 0.001% bromophenol blue) for the pattern of protein expression before and after induction of GST-fusion proteins.

Purification of GST-Fusion Proteins Using Nonionic Detergent

The GST-fusion proteins were expressed using the same procedure as described above. The culture was collected by centrifugation for 20 min at 2000 rpm. The bacterial pellet was resuspended in 1 ml of EBC buffer [50 mM Tris-HCl, pH 8.0, 120 mM NaCl, and 0.5% IGEPAL CA630 (same with Nonidet P-40, Sigma)] containing 1 mM dithiothreitol (DTT) and 1 μg/ml of aprotinin and leupeptin (Sigma). The suspension was incubated for 15 min on ice and the cells were lysed on ice by 3 cycles of sonication for 10 sec. Cellular debris was removed by centrifugation at 12,000 rpm in a microcentrifuge for 5 min at 4°C. The supernatant was collected for the soluble fraction of protein preparations and stored at -70°C.

Purification of GST-Fusion Proteins Using Glutathione-Sepharose Beads

To purify GST-fusion proteins from the soluble fraction of protein preparations, 200 μl of lysate was selectively bound to 20 μl of glutathione-Sepharose beads (50% slurry, Pharmacia Biotech) pre-equilibrated in EBC buffer by incubation for 30 min at room temperature with gentle rocking. The beads were collected by centrifugation and washed with a 20-fold volume of EBC. The protein-bound Sepharose beads were resuspended in 20 μl of 2× SDS loading buffer.

Analysis of Proteins Using SDS-Polyacrylamide Gel Electrophoresis (PAGE)

The proteins were analyzed on 15% SDS-polyacrylamide gels (4% stacking gel containing 0.12 M Tris-HCl, pH 6.8 and 0.1% SDS: 15% separating gel containing 0.375 M Tris-HCl, pH 8.8, and 0.1% SDS; acrylamide: bis-acrylamide of 30:0.8) which were run in 25 mM Tris and 250 mM Glycine buffer, pH 8.3, containing 0.1% SDS at room temperature [1]. Detection of proteins by Coomassie blue staining [0.05% Coomassie Brilliant blue R-250 (Sigma), 50% methanol, and 10% acetic acid] was performed by standard methods [1].

RESULTS

Aggregation of GST-MJD Fusion Proteins Containing Polyglutamine

Pathogenesis of several neurodegenerative disorders including Machado-Joseph disease is associated with the aggregation of expanded glutamine repeats within their proteins. For study of the formation of polyglutamine aggregates in *E. coli*, we utilized the pGEX expression system. Genomic DNAs containing a portion of MJD gene that were derived from normal (8 CAG repeats) and expanded alleles (65 repeats) of a Korean MJD patient were amplified by the PCR with primers, F-MJD and R-MJD (see Materials and Methods and Fig. 1). Since it has been shown previously that the solubility of certain proteins can be enhanced by the addition of the GST [20], plasmids that encode GST-fusion proteins containing polyglutamine residues and the flanking amino acids joined to the carboxyl terminus of glutathione-S-transferase (GST) were constructed (Fig. 1) and expressed in *E. coli* strain XA 90 by induction with IPTG, as described under Materials and Methods. It was previously reported that high levels of the GST-fusion proteins could be expressed in this protease deficient strain. After induction with IPTG, the cells were lysed by SDS buffer and run on a polyacrylamide gel. As shown in Fig. 2, the normal GST-$(Gln)_8$ and expanded $(Gln)_{65}$ proteins accumulated to similar levels in *E. coli* culture (lanes 2 and 7). The GST-$(Gln)_8$ and GST-$(Gln)_{65}$ proteins have a calculated molecular mass of 33.4 kDa and 39.6 kDa, respectively. The GST-$(Gln)_{65}$ protein, however, migrates anomalously in SDS-polyacrylamide gels at approximately 46 kDa. Previously, Ikeda *et al.* [8] showed that 79 polyglutamine repeats migrated at a position higher than its expected size.

Since glutathione bead binding was used to assess the aggregation of the *E. coli*-expressed GST-fusion proteins, GST-fusion proteins were purified from cell lysates under non-denaturing conditions by selective binding to glutathione-Sepharose beads. After lysis of the cultures by sonication, the proteins were run on a polyacrylamide

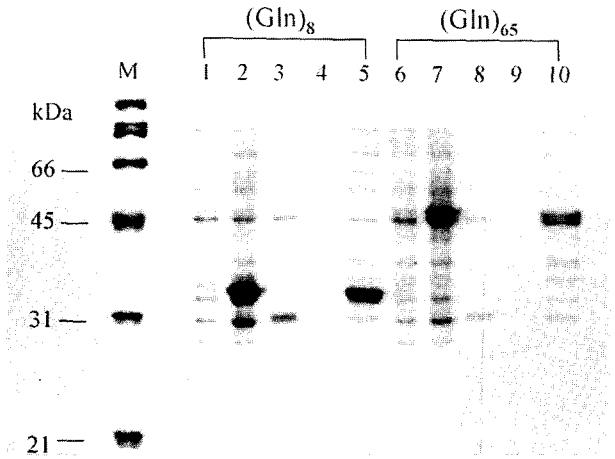

Fig. 2. SDS-PAGE of proteins containing polyglutamine expressed as GST-fusion proteins in *E. coli.*

Proteins were overexpressed and solubilized as detailed in Materials and Methods. Proteins were analyzed on a 15% SDS-polyacrylamide gel which was stained with Coomassie Brilliant blue. Lane M contains protein markers (Bio-Rad); lanes 1, 2, 6, and 7 are whole-cell pellets of *E. coli* cultures before (lanes 1 and 6) and after (lanes 2 and 7) IPTG induction of GST-$(Gln)_8$ and -$(Gln)_{65}$ proteins, which were disrupted by SDS; lanes 3 and 8 are whole-lysates prepared by sonication of the cultures; lanes 4 and 9 are GST-$(Gln)_8$ and GST-$(Gln)_{65}$ proteins selectively bound to glutathione-Sepharose beads in lanes 3 and 8, respectively; lanes 5 and 10 are proteins that remain in the precipitated portion of preparations plus whole-cell pellets that were not compleletely lysed by sonication.

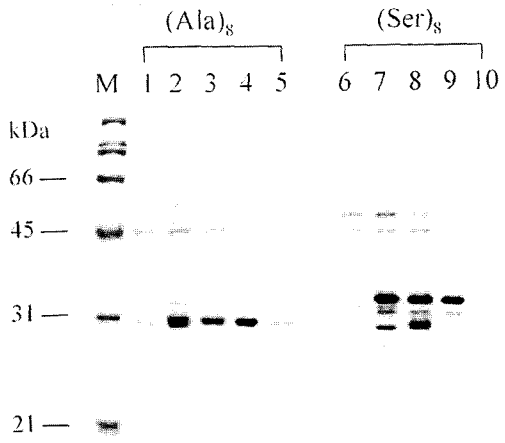

Fig. 3. Overexpression of GST-$(Ala)_8$ and GST-$(Ser)_8$ proteins in *E. coli.*

Proteins were analyzed on a 15% SDS-polyacrylamide gel which was stained with Coomassie Brilliant blue. All lanes are the same order as described in Fig. 1. Lane M contains protein markers (Bio-Rad); lanes 1, 2, 6, and 7 are whole-cell pellets of *E. coli* cultures before (lanes 1 and 6) and after (lanes 2 and 7) IPTG induction of GST-$(Gln)_8$ and -$(Gln)_{65}$ proteins, which were disrupted by SDS; lanes 3 and 8 are whole-lysates prepared by sonication of the cultures; lanes 4 and 9 are GST-$(Gln)_8$ and GST-$(Gln)_{65}$ proteins selectively bound to glutathione-Sepharose beads in lanes 3 and 8, respectively; lanes 5 and 10 are proteins that remain in the precipitated portion of preparations plus whole-cell pellets that were not compleletely lysed by sonication.

gel. Surprisingly, the band of the normal GST-$(Gln)_8$ as well as that of expanded GST-$(Gln)_{65}$ proteins in the soluble fraction disappeared (Fig. 2, lanes 3, 4, 8, and 9). Our further study showed that GST-fusion proteins containing polyglutamine residues were precipitated with cell debris (Fig. 2, lanes 5 and 10). These results indicate that GST-fusion proteins containing polyglutamine residues form protein aggregates or inclusion bodies.

GST-Fusion Proteins Containing Polyalanines and Polyserines Are Soluble

In order to examine whether polyglutamine repeat residues within the protein were responsible for the formation of protein aggregates, we generated +1 and +2 frameshift mutants of polyglutamine by the PCR, resulting in proteins containing the same length of polyserine and polyalanine residues, respectively (see Materials and Methods and Fig. 1). The length of the frameshift mutant proteins was shorter than that of the wild-type protein containing polyglutamine residues, because termination codons were created by the +1 or +2 frameshifting in the coding region of the 3' flanking sequence of the CAG repeat sequence (Fig. 1). As shown in Fig. 3, the GST-$(Ala)_8$ and GST-$(Ser)_8$ fusion proteins were present in cell lysates at approximately similar levels (lanes 2 and 7). The GST-$(Ala)_8$ and GST-$(Ser)_8$ proteins have a calculated molecular mass of 29.1 kDa and 30.6 kDa, respectively. Both GST-fusion proteins, however, migrate slightly higher than that of the calculated molecular mass in the SDS-polyacrylamide gel (Fig. 3). As shown in lanes 3, 4, 8, and 9, the frameshift mutants of the GST-fusion proteins were present in the soluble fraction after the solubilization of cell pellets by sonication, indicating that the overexpressed GST-fusion proteins containing polyalanine and polyserine residues are highly soluble. These results indicate that the GST-fusion proteins containing polyalanine and polyserine residues in *E. coli* system were less susceptible to aggregation than the GST-polyglutamine fusion proteins. Our results imply that the insoluble protein aggregates or inclusion bodies of GST fusion proteins containing polyglutamines were enhanced by the presence of polyglutamine residues themselves in the GST-fusion proteins.

Investigaion of Solubility of Other GST-Fusion Proteins

We further examined whether other GST-fusion proteins can form aggregates under our protein preparation conditions. Plasmids that encode five additional GST-fusion proteins including α-synuclein (the precursor protein of the non-Aβ component of Alzheimer's disease amyloid), NAC (the non-Aβ component of Alzheimer's disease amyloid), anti-Xpress, Tat-1, and Tat-2 were constructed as described in Materials and Methods. The GST-fusion

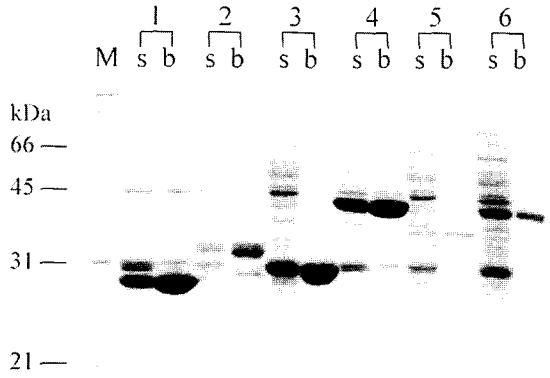

Fig. 4. Overexpression of other GST-fusion proteins in *E. coli.*

Proteins were analyzed on a 15% SDS-polyacrylamide gel which was stained with Coomassie Brilliant blue. s lanes are whole-lysates prepared by sonication of the cultures and b lanes are GST-fusion proteins selectively bound to glutathione-Sepharose beads; GST (lane 1), GST-α-synuclein (lane 2), GST-NAC (lane 3), GST-anti-Xpress (lane 4), GST-Tat-1 (lane 5), and GST-Tat-2 (lane 6).

proteins were expressed and purified from cell lysates under non-denaturing condition by selective binding to glutathione-Sepharose beads. All GST-fusion proteins were detected in soluble fraction at approximately ten-fold higher levels than GST-$(Gln)_8$ and GST-$(GST)_{65}$ fusion proteins (Fig. 4) under our protein preparation conditions. These data further support the hypothesis that the formation of protein aggregates in GST-polyglutamine proteins is specifically mediated by the polyglutamine repeat sequence within their protein structure.

DISCUSSION

This study shows that poly$(Gln)_8$ in the normal range as well as poly$(Gln)_{65}$ in the pathogenic range enhances the formation of insoluble protein aggregates. However, the GST-$(Ala)_8$ and GST-$(Ser)_8$ proteins could be readily expressed and purified in soluble forms. Five additional GST-fusion proteins were detected in the soluble fraction at approximately ten-fold higher levels than GST-$(Gln)_8$ and GST-$(Gln)_{65}$ fusion proteins under our protein preparation conditions. Taken together, these results indicate that the formation of protein aggregates in GST-polyglutamine proteins is mediated by the polyglutamine repeat residues within their protein structure.

Scherzinger *et al.* [19] suggested that the elongation of the polyglutamine repeat beyond a certain length may lead to hydrogen-bonded hairpins in the polyglutamine stretch resulting in protein aggregates. Our results showed that GST-fusion proteins containing 8 polyglutamine repeats form protein aggregates. We are not sure at this point that the 8 polyglutamine repeats are long enough to form hairpins. Our explanation for the aggregation of 8 polyglutamine repeats is as follows. First, the GST-fusion proteins exist as a dimer with two expanded polyglutamine residues that form antiparallel β-strands with the polyglutamine residues from other dimer GST-fusion proteins, resulting in the protein aggregates. Secondly, the presence of polyglutamine sequences may hinder the correct folding of GST-fusion proteins containing polyglutamine repeats, resulting in the aggregates of GST-fusion proteins. Third, the segment of the MJD protein flanking $(Gln)_n$ could have a role in the aggregation, although it is not likely.

Martindale *et al.* [11] showed that the frequency of aggregates is modulated by polyglutamine length as well as the length of the protein flanking the polyglutamine. Although the glutamine-rich region in the GST-fusion proteins is located among 16 additional residues in the carboxyl terminus of GST and 58 flanking residues of the carboxyl terminal portion of the MJD protein, GST-polyglutamine proteins appear to be extremely sensitive to aggregation. Its precise mechanism of action remains to be elucidated.

Our results demonstrate that the insolubility of GST-fusion proteins containing polyglutamine is increased by a role of polyglutamine residues. It has been previously thought that aggregates in brain cells produce an abnormal gain of function that in turn may induce neuronal degeneration and cell death. Therefore, development of inhibitors that prevent the aggregation might aid in therapeutic approaches of neurodegenerative diseases. Our study of the formation of aggregates in *E. coli* may contribute to a better understanding of the molecular mechanism of the formation of insoluble protein aggregates in neurodegenerative disorders and the development of preventative strategies.

Acknowledgments

We thank Sung Jo Kim for preparing the figures. This work was partially supported by a grant from the Ministry of Science and Technology, Korea (97-N1-2-03-A-02) and the Ministry of Education (1998-019-D00056).

REFERENCES

1. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1989. In *Current Protocols in Molecular Biology*, Chap. 16, Wiley, NY, U.S.A.
2. Burke, J. R., J. J. Enghild, M. E. Martin, Y.-S. Jou, R. M. Myers, A. D. Roses, J. M. Vance, and W. J. Strittmatter. 1996. Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nature Med.* **2:** 347–350.

3. Colicelli, J. and H. Lehrach. 1997. HIP-1: A huntingtin interacting protein isolated by the yeast two-hybrid system. *Hum. Mol. Genet.* **6:** 487–495.
4. Davies, S. W., M. Turmaine, B. A. Cozens, M. DiFiglia, A. H. Sharp, C. A. Ross, E. Scherzinger, E. E. Wanker, L. Mangiarini, and G. P. Bates. 1997. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90:** 537–548.
5. Dupret, J. M., G. H. Goodfellow, S. A. Janezic, and D. M. Grant. 1994. Structure-function studies of human arylamine N-acetyltransferases NAT1 and NAT2: Functional analysis of recombinant NAT1/NAT2 chimeras expressed in *E. coli. J. Biol. Chem.* **269:** 26830–26835.
6. Herrmann, C. H. and A. P. Rice. 1993. Specific interaction of the human immunodeficiency virus Tat proteins with a cellular protein kinase. *Virology* **197:** 601–608.
7. Huntington's Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is unstable on Huntington's disease chromosomes. *Cell* **72:** 971–983.
8. Ikeda, H., M. Yamaguchi, S. Sugai, Y. Aze, S. Narumiya, and A. Kakizuka. 1996. Expanded polyglutamine in the Machado-Joseph disease protein induces cell death *in vitro* and *in vivo. Nature Genet.* **13:** 196–202.
9. Kahlem, P., C. Terre, H. Green, and P. Djian. 1996. Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: Relevance to diseases of the nervous system. *Proc. Natl. Acad. Sci. USA* **93:** 14580–14585.
10. Kalchman, M. A., H. B. Koide, K. McCutcheon, R. K. Graham, K. Nichol, K. Nishiyama, P. Kazemi-Esfariani, F. C. Lynn, C. Wellington, and M. Metzler. 1997. HIP1, a human homologue of *S. cerevisiae* Sla2p, interacts with membrane-associated huntingtin in the brain. *Nature Genet.* **16:** 44–53.
11. Martindale, D., A. Hackam, A. Wieczorek, L. Ellerby, C. Wellington, K. McCutcheon, R. Singaraja, P. Kazemi-Esfarjani, R. Devon, S. U. Kim, D. E. Bredson, F. Tufaro, and M. R. Hayden. 1998. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nature Genet.* **18:** 150–154.
12. Matilla, A., B. T. Koshy, C. J. Cummings, T. Isobe, H. T. Orr, and H. Y. Zoghbi. 1997. The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. *Nature* **389:** 974–978.
13. Ordway, J. M., S. Tallaksen-Greene, C.-A. Gutekunst, E. M. Bernstein, J. A. Cearley, H. W. Wiener, L. S. Dure, R. Lindsley, S. M. Hersch, R. S. Jope, R. L. Albin, and P. J. Detloff. 1997. Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell* **91:** 753–763.
14. Paulson, H. L., S. S. Das, P. B. Crino, M. K. Perez, S. C. Patel, D. Gotsdiner, K. H. Fischbeck, and R. N. Pittman. 1997. Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. *Ann. Neurol.* **41:** 453–462.
15. Reddy, P. S. and D. E. Housman. 1997. The complex pathology of trinucleotide repeats. *Curr. Opin. Cell Biol.* **9:** 364–372.
16. Rhim, H., F. Chan, C. O. Echetebu, and A. P. Rice. 1993. Rapid purification of monomer HIV-2 Tat protein expressed in *E. coli. Protein Expression Purif.* **4:** 24–31.
17. Rhim, H. and A. P. Rice. 1993. TAR RNA binding properties and relative transactivation activities of human immunodeficiency virus type 1 and 2 Tat proteins. *J. Virol.* **67:** 1110–1121.
18. Ross, C. A. 1995. When more is less: Pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* **15:** 493–496.
19. Scherzinger, E., R. Lurz, M. Turmaine, L. Mangiarini, B. Holenbach, R. Hasenbank, G. P. Bates, S. W. Davies, H. Lehrach, and E. E. Wanker. 1997. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo. Cell* **90:** 549–558.
20. Smith, D. B. and K. S. Johnson 1988. Single-step purification of peptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* **67:** 31–40.
21. Wanker, E. E., C. Rovira, E. Scherzinger, R. Hasenbank, S. Walter, D. Tait, and M. F. Perutz. 1996. Glutamine repeats and inherited neurodegenerative diseases: Molecular aspects. *Curr. Opin. Struct. Biol.* **6:** 848–858.