Intra- and Intermolecular β-Pleated Sheet Formation in Glutamine-repeat Inserted Myoglobin as a Model for Polyglutamine Diseases

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An aberrant structure of the expanded polyglutamine might be involved in the formation of aggregates in CAG repeat diseases. To elucidate structural properties of the expanded polyglutamine, we prepared sperm whale myoglobin (Mb) mutants, in which 12, 28, 35, and 50 repeats of glutamine were inserted at the corner between the C and D helices (Gln12, Gln28, Gln35, and Gln50, respectively). Circular dichroism and IR spectroscopies showed that the expanded polyglutamine, which was recognized by the monoclonal antibody 1C2 in Gln28, Gln35, and Gln50 Mb forms an antiparallel β-pleated sheet structure. Mutant Gln50 Mb aggregates were found to comprise an intermolecular antiparallel β-pleated sheet. Fluorescence together with 1H NMR spectra revealed partial unfolding of the protein surface in Gln35 and Gln50 Mb, although the structural changes in the protein core were rather small. The present results indicate that the fluctuating β-pleated sheet of the expanded polyglutamine exposed on the protein surface facilitates the formation of aggregates through intermolecular interactions. The present study has first established and characterized structural properties of a molecular model for polyglutamine diseases in which various lengths of polyglutamine including a pathologically expanded glutamine repeat were inserted into a structurally known protein.

Neurodegenerative diseases such as Huntington’s disease (HD)1 and hereditary spinocerebellar ataxia (SCA) are linked to abnormally expanded CAG repeats in the coding regions of responsible genes and are categorized as CAG repeat diseases. The expanded CAG repeat is translated into an extended polyglutamine, which confers deleterious novel functions on the mutant proteins. A hallmark of CAG repeat diseases is the development of intracellular protein aggregates in vulnerable neurons. The aggregate formation is closely related to expansion of the CAG repeat, namely expanded polyglutamine in the affected proteins. Trottier et al. (1) reported that the expanded polyglutamine in the proteins implicated with HD, SCA1, and SCA3 are selectively recognized by the monoclonal antibody 1C2. The finding that 1C2 reacts with polyglutamine only of the pathological repeat length provided a structural basis for the notion that the expanded polyglutamine forms an abnormal structure, resulting in the formation of aggregates and possibly inducing neuronal cell death.

Although the importance of understanding the structure of the expanded polyglutamine has been emphasized, the highly unstable proteins tethering an expanded glutamine repeat have prevented isolation of the affected proteins. For instance, proteolytic cleavage of glutathione S-transferase (GST) from GST-huntingtin(exon1) fusion protein rapidly leads to the formation of insoluble aggregate (2). To elucidate the structure of the expanded glutamine repeat, polyglutamine peptides were synthesized. Poly-L-glutamine, however, is insoluble in water, and even short oligoglutamine chains form aggregates in solution to form viscous gels (3). Perutz et al. (3) found, using circular dichroism (CD) spectroscopy, that 15 glutamine repeats form a β-sheet structure and proposed from results of computer simulation that two paired antiparallel β-strands of poly-L-glutamine form a “polar zipper” structure. On the other hand, Altschuler et al. (4) showed that 17 glutamine repeats do not adopt a β-sheet but form a random coil structure. The discrepancy in these results may have been caused by differing composition of the polar residues such as Asp and Lys added to both ends of the polyglutamine peptide. Although the polar residues are essential to increase the solubility of the polyglutamine peptide, the introduction of polar residues may induce some artificial effects on the structure of polyglutamine.

Another strategy to explore the structure of polyglutamine is to insert a polyglutamine molecule into a specific region of a stable protein. Mutant mice carrying an expanded CAG repeat introduced into the mouse hypoxanthine phosphoribosyltransferase (Hprt) gene developed a phenotype similar to that of human CAG repeat diseases, suggesting that expanded CAG repeats do not necessarily have to be located within the genes that already have a CAG repeat (5). In other words, any protein having an expanded glutamine repeat can be a model for the gene products of CAG repeat diseases. Stott et al. (6) characterized a chymotrypsin inhibitor 2 (CI2) mutant containing an inserted 10 glutamine repeat. CD spectroscopy showed that the CI2 mutant forms a type I β-turn in a monomer state and a β-sheet in dimeric and trimeric states. The x-ray crystal struc-
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ture of the polyglutamine moiety in the mutant has not been solved due to disorder of the polyglutamine tract (7).

The glutamine repeats examined in these studies using polyglutamine peptides and the CI2 mutant are much shorter than the pathological repeat in CAG repeat diseases (~35 glutamine repeats). Furthermore, the reactivities of both polyglutamine peptides and the CI2 mutant with the 1C2 antibody have not been reported, although it is crucial to examine the polyglutamine structure that is recognized by 1C2. Therefore, a putative aberrant structure of the expanded polyglutamine has remained unclear. In addition, the effects of the expanded glutamine repeat on protein structure and stability have never been investigated. These influences might also be crucial for the aggregate formation since the expansion of polyglutamine may induce serious structural changes in the other parts of the protein, which would facilitate the formation of aggregates.

In the present study, we inserted an expanded glutamine repeat into sperm whale myoglobin (SW Mb) since SW Mb is one of the most stable proteins, and it is highly soluble. 12, 28, 35, or 50 repeats of glutamine were inserted at the corner between the C and D helices in Mb (Gln12, Gln28, Gln35, and Gln50, respectively). The number of glutamine repeats in HD patients is more than 36. Thus, 12 and 28 glutamine repeats are nonpathological and the 50 glutamine repeat is pathological, whereas the 35 glutamine repeat is located near the border between normal individuals and HD patients. We expressed these mutant Msbs in Escherichia coli and examined the structures of the mutants as well as the effects of expanded glutamine repeat on protein stability using various types of spectroscopy including CD, infrared (IR), and 1H NMR.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Protein Expression—pSI and Spel sites were introduced at the positions of Ser-44 and Arg-47, respectively, in the pUC19-SW Mb expression vector constructed by Springer and Sli-gar (8). We obtained various lengths of CAG repeats (12, 28, 35, or 50) from the Machado-Joseph disease (MJD) gene. To assist protein folding, several codons from the MJD gene were added to both ends of the CAG repeat. The DNA fragments including CAG repeats flanked by pSI and SpeI sites were amplified by polymerase chain reaction and ligated into the pUC19-SW Mb expression vector.

TB1 was used as a host E. coli strain for expression of the pUC19-SW Mb vector. Recombinant SW Mb was expressed and purified according to the method of Springer and Sli-gar (8). Purity of the final sample checked by SDS-polyacrylamide gel electrophoresis showed a single protein band. The BZ (ratio of the absorbance at 408 nm to that at 280 nm) values of purified ferric wild-type is 5.0, whereas those of the mutant Msbs (Gln12, Gln28, and Gln35) were above 4.0. The BZ value of the Gln50 Mb mutant was more than 3.3. The extinction coefficient, 157 mM⁻¹ cm⁻¹, at the Soret band in a UV-visible spectrum determined the concentration of ferric Msbs.

Immunoblotting—A polyclonal antibody for human myoglobin (Chemicon) and a monoclonal antibody for polyglutamine (1C2/Chemicon) were used for immunoblotting.

Spectroscopies—Far-UV CD spectra of ferric Msbs (3.1 μM) in 50 mM potassium phosphate buffer, pH 7.0 were measured using a JASCO J-720 spectropolarimeter at 25 °C. The spectra were an average of 16 scans recorded at a speed of 100 nm/min and a resolution of 0.2 nm. 1H NMR spectra were recorded using a BRUKER Avance DRX600 spectrometer at 25 °C. A LOSAT pulse sequence was used with 64,000 average of 4096 scans recorded at a speed of 15 scans/min and a resolution of 2 cm⁻¹.

Congo Red Binding—5 μl of 200 μM Mb, which was incubated at 37 °C for 5 days, was mixed with 100 μl of 2.5 μM Congo red solution and left at ambient temperature for 30 minutes. The formation of UV-visible spectrum of the mixture was measured on a Shimadzu UV-2400 spectrophotometer.

Electron Microscopy—150 μM Mb was incubated at 37 °C for 2 weeks and adsorbed onto carbon-coated 400-mesh copper grids followed by 2% paraformaldehyde, 2% glutaraldehyde in 100 mM sodium phosphate buffer. The sample was negatively stained with 2% sodium phospho-tungstic acid. Images were recorded on a LEO 912AB electron micro-scope (LEO, Cambridge, UK) at a magnification of ×50,000.

RESULTS

Design and Preparation of Sperm Whale Myoglobin Mutants Containing an Inserted Glutamine Repeat—Sperm whale Mb is a single polypeptide chain of 153 residues consisting of eight α-helices from A to H that occupy 80% of the total residues and that does not contain any β-sheet structures (Fig. 1A). The eight α-helices are packed tightly, and a heme prosthetic group located between the E and F helices reinforces their packing, which renders the protein quite stable. The x-ray crystal structure of SW Mb shows that the loop between the C and D helices (C-D corner) is exposed to solvent and that the temperature factors of the residues in the loop region are higher than any other residues, suggesting that the C-D corner is quite flexible and does not contribute to the stability of the protein (10).

Therefore, a polyglutamine chain inserted at the corner between the C and D helices in Mb should form its own inherent structure. In this study, we prepared SW Mb mutants, in which 12, 28, 35, or 50 repeats of glutamine were inserted at the corner between the C and D helices (Gln12, Gln28, Gln35, and Gln50, respectively). To assist formation of the intrinsic structure of the insert, we added several residues from the MJD protein as a linker to both ends of the polyglutamine stretch (Fig. 1A).

To confirm correct folding of the mutant Msbs, UV-visible spectra of ferric mutant Msbs were measured. The spectral features of all mutant Msbs were quite similar to that of wild-type Mb (data not shown). This result indicates that the mutant Msbs were properly folded regardless of the inserted glutamine repeat.

Characterization of Polyglutamine and Aggregates in Mutant Msbs—We examined whether or not the polyglutamine inserted into SW Mb also forms the abnormal structure as do the proteins for CAG repeat diseases by immunoblotting with 1C2 antibody. While the amounts of wild-type and mutant Msbs were almost identical as indicated by the immunoblot using the Mb antibody, the reactivity of mutant Msbs using 1C2 clearly depended on the length of the glutamine repeat (Fig. 1, B and C). A specific band was detected in the lanes containing Gln28, Gln35, and Gln50 Msbs, of which Gln50 reacted most intensely against 1C2 antibody. In contrast, neither Gln12 nor the wild-type Mb developed bands. We concluded that the mutant Msbs formed a polyglutamine structure similar to that of the proteins for CAG repeat diseases, indicating that the mutant Mb is a good model protein for CAG repeat diseases.

To examine the formation of aggregates, 100 μM Gln50 was...
incubated at 37 °C for 1, 2, and 3 days, and the same aliquot of the “aged” Gln50 solution was separated by SDS-polyacrylamide gel electrophoresis followed by immunoblotting using Mb and 1C2 antibodies (Fig. 1, D and E). Mb antibody stained a gel top as well as a soluble monomeric Gln50 at 26 kDa, whereas 1C2 antibody did not react with the aggregates at the gel top but did react with the soluble form. The staining of the gel top and the band at 26 kDa indicates the aggregated and soluble monomeric forms of the Gln50 mutant, respectively.

aggregates (Fig. 1, D and E). We further investigated whether Gln50 oligomers were transiently present in the process of aggregate formation using analytical ultracentrifugation. 100 μM Gln50 was centrifuged at 60,000 rpm at 20 °C, and the absorbances at 280 and 400 nm were monitored. In this experiment, only monomeric and aggregated forms of Gln50 were detected, whereas any stable oligomers were not observed during the formation of aggregates (data not shown).

Next, structural features of the inserted polyglutamine in the mutant Mbs were investigated. We initially examined the secondary structure of the inserted polyglutamine using CD spectroscopy. Fig. 2A shows the CD spectra of ferric wild-type and mutant Mbs in the far-UV region. The spectrum of wild-type Mb, which contains only α-helices, was typical of an α-helix with two negative peaks at 209 and 222 nm (11). Although the spectral shapes of the mutant Mbs were similar to those of wild-type Mb, a negative molar ellipticity at 208 nm was slightly enhanced upon elongation of the glutamine repeat. Subtraction of the wild-type spectrum from the spectra of the mutants revealed the CD spectra of the polyglutamine inserts in the mutant Mbs (Fig. 2B). The molar ellipticity at 206 and 222 nm in the difference CD spectra decreased as the glutamine repeats were extended.

We then examined the secondary structure of the glutamine repeat inserted into SW Mb by FT-IR spectroscopy. Fig. 3A shows FT-IR spectra in the amide I region of wild-type and mutant Mbs. We observed a large peak centered at 1650 cm⁻¹ in the spectrum of wild-type Mb that has been previously assigned to the amide I band of α-helix in carbon monoxide-ligated myoglobin (12). Although the IR spectra of mutant Mbs resembled that of the wild-type, slight but significant differences were detected. These were clarified by the difference spectra as shown in Fig. 3B. A positive peak was observed at 1634 cm⁻¹ in the difference spectra of mutant Mbs, and its intensity increased upon expansion of the glutamine repeat. An IR spectrum of a typical β-sheet-rich protein, β-lactoglobulin, showed a peak at 1635 cm⁻¹ and has been previously assigned to the amide I band of an intramolecular β-sheet (13). Therefore, the present data demonstrated that the polyglutamine inserted in Mb forms an intramolecular β-pleated sheet. In addition, we found that the peaks at 1616, 1674, and 1685 cm⁻¹ appeared only in the difference spectrum of Gln50 Mb. A previous study assigned the pair of the peaks at 1616 and 1685 cm⁻¹ to an intermolecular antiparallel β-pleated sheet structure. The spectrum of “aged Gln50”, which was incubated at 37 °C, also showed the pair of the peaks, indicating slower formation of the intermolecular β-sheet ag-
The IR spectrum of wild-type Mb from those of mutant Mbs in the amide I region.

Other Regions of Mb

We examined the effects of expanding the glutamine repeat (14) (Fig. 5) on other regions of the protein. Since the heme group in SW Mb has paramagnetism and is located in the interior of the protein, we investigated the structural effects of polyglutamine on the protein core, the heme, and the heme environmental structure using paramagnetic 1H NMR spectroscopy. The resonances position for heme peripheral methyl groups at 90, 84, 72, and 53 ppm (8-, 5-, 3-, and 1-methyl group) as well as for N$_2$H of the proximal His (His-93) at 103 ppm were not changed by the insertion and expansion of the glutamine repeat (14) (Fig. 5A).

To further understand the heme environmental structure, we measured the 1H NMR spectra of cyanide-ligated Mbs. Several amino acid protons surrounding the heme group and heme peripheral protons have been assigned in the low field region. While 8-, 5-, and 1-methyl protons of the heme group were observed at 27.3, 18.7, and 12.9 ppm, His-93 N$_2$H, Phe-43 C$_2$H, and His-93 N$_2$H were detected at 21.3, 17.2, and 13.8 ppm, respectively, in the spectrum of wild-type Mb (15) (Fig. 5B). The spectral profile was not affected by introducing a glutamine repeat into the C-D corner of Mb. In the high field region, a broad signal at -9.9 ppm that is assigned to Ile-99 C$_2$H was not changed in the mutant Mbs (data not shown). The lesser spectral changes in the heme and heme environment by glutamine repeats were also confirmed by the resonance Raman spectra of ferric and ferrous Mbs (data not shown).

To examine the effects of the inserted glutamine repeat on the protein surface, we measured fluorescence by tyrosine and tryptophan residues with excitation at 280 nm. Three tyrosines (Tyr-103, Tyr-146, and Tyr-151) and one tryptophan (Trp-7) are located at the protein surface, while one tryptophan (Trp-14) is buried inside the protein. Since the fluorescence from Trp-14 is readily quenched by the heme group, the emission from the three Tyr residues and one Trp-7 contribute to the fluorescence spectra (16). The emission maximum in fluorescence spectra is roughly correlated with the distance and orientation between heme and Tyr/Trp in heme proteins (17). Since the geometry of the heme group was not altered by the inserted glutamine repeat (Fig. 5), the emission maximum in this study provides structural information on the three Tyr and one Trp residues located on the protein surface. The emission maximum was 331 nm in the fluorescence spectrum of wild-type Mb, while the wavelength at the peak showed a redshift from 331 to 334, 342, 344, and 347 nm in the spectra of Gln$_{12}$, Gln$_{28}$, Gln$_{35}$, and Gln$_{50}$ mutant Mbs, respectively. The redshift of the emission maximum is caused by exposing Tyr and/or Trp to the solvent (17).

Effects of Insertion and Expansion of Polyglutamine on Protein Stability—The previous result suggests that expanding the glutamine repeat induced a fluctuation of the protein surface, which might affect protein stability. Thus, the effects of the
expansion as well as the insertion of polyglutamine on the stability of the protein were examined. We monitored the molar ellipticity at 222 nm in CD spectra and the absorbance at the Soret band in UV-visible spectra during unfolding and refolding of wild-type and mutant Mbs and evaluated the stability of the overall or heme environmental structures, respectively. To unfold and refold holo-Mbs reversibly, we used the cyano form of holo-Mbs and GdmCl as a denaturant (9). Fig. 6 shows the GdmCl-induced unfolding processes of wild-type and mutant cyano-Mbs monitored by the molar ellipticity at 222 nm (Fig. 6A) and the absorbance at 419 nm (Fig. 6B). The transition curves under these conditions confirmed the two states and reversibility (data not shown). From the sigmoidal curves, we calculated the concentration of GdmCl at the midpoint of the unfolding/refolding transition (Cm), which is an index of protein stability (9). The decrease in Cm values obtained from both CD and UV-visible spectra of the mutant Mbs relative to those of wild-type Mb indicated that the insertion of the glutamine repeat destabilized both overall and heme environmental structures. We then examined the effects of extending the glutamine repeat on protein stability by plotting Cm values against the number of glutamine repeats (Fig. 6C). In Cm values calculated from the CD spectra, the decreases in Cm values of the mutant Mbs were dependent on the number of glutamine repeats. The extent of the decrease in Cm of Gln15 and Gln50 mutants relative to that of wild-type Mb was unambiguously enhanced compared with that of the Gln12 and Gln28 mutants. In contrast, the Cm values from UV-visible spectra did not show substantial changes between mutant Mbs, indicating that the stability of the heme environment was insensitive to up to about 50 glutamine repeats.

**DISCUSSION**

**Structure of Inserted Glutamine Repeat in Mutant Mbs—**The formation of the antiparallel β-sheet of the expanded polyglutamine was confirmed by the IR spectra because the peak at 1634 cm⁻¹ in the difference IR spectra revealed that the 28, 35, and 50 glutamine repeats inserted into the mutant Mbs formed an intramolecular β-sheet (13). The formation of the antiparallel β-sheet is consistent with the results of the CD spectra. The difference CD spectra with negative peaks at 206 and 223 nm, which correspond to the spectra of the inserted polyglutamine, closely resemble that of the type I β-turn (20). Since molar ellipticity of the β-sheet is approximately 2-fold smaller than that of the type I β-turn (20), the CD spectra of polyglutamine would be dominated by that of the type I β-turn rather than the β-sheet. The peak at 1674 cm⁻¹ in the difference IR spectra of Gln12 Mb also supports the presence of the β-turn in the expanded polyglutamine (12, 13). On the other hand, the IR spectrum of Gln12 did not show a peak at 1634 cm⁻¹, indicating that a short glutamine repeat did not form an intramolecular β-sheet or that such a short β-sheet of 12 glutamines was undetectable in the difference IR spectrum.

Gln50 Mb forms an intramolecular β-sheet, and the Gln50 aggregates have an intramolecular antiparallel β-sheet as evidenced by IR spectra (Fig. 3B). Since wild-type myoglobin does not possess any β-sheet structures, it is likely that the β-sheet formed by the expanded polyglutamine induced the formation of aggregates by its intermolecular interactions. The failure to detect the Gln50 aggregates at the gel top by 1C2 antibody demonstrated that the polyglutamine repeat inserted in myoglobin forms a core of the aggregates. The Gln50 aggregates were found to contain amyloid fibrils as shown by Congo red binding and birefringence. The presence of amyloid fibers agrees well with the huntingtin aggregates in R6/2 HD model mice and the GST-huntingtin(exon1) fusion protein aggregates (2, 21).

Despite the close similarity of the mutant Mbs to proteins for CAG repeat diseases, we found that one difference was certainly observed between them. Although 28 repeats of glutamine in huntingtin and SCA1 proteins were not detected by 1C2 antibody, the 28-repeat length was enough to react with 1C2 in the mutant Mb (1). This inconsistency might be caused by the fact that the polyglutamine in mutant Mbs has been designed to be located entirely on the protein surface. The location of the whole polyglutamine insert on the protein surface in the Gln15 mutant would improve the reactivity with 1C2 antibody. Conversely, the difference in the reactivity against 1C2 between the mutant Mbs and proteins for CAG repeat diseases implies that the β-strand of polyglutamine in the latter is partially covered with the other structures and buried in the protein. Since the border of the glutamine repeat between normal individuals and patients varies among CAG repeat diseases, this study suggests that the extent to which polyglutamine is exposed to solvent determines the repeat number of glutamine at the boundary between normal and affected individuals.

The length dependence in the reaction between 1C2 and polyglutamine suggests that 1C2 detects a unique conformation that requires a minimum length of polyglutamine and that it is stabilized by further lengthening of glutamine repeat. Our data suggest that 1C2 recognizes the type I β-turn of polyglutamine that appeared on the protein surface after expansion of the glutamine repeat. The β-turn in the polyglutamine β-strand would be stabilized by the expansion of the glutamine repeat since participation of more glutamine residues in the formation of the β-sheet increases the rigidity of the β-turn as well as of the β-strand itself. Thus, 1C2 would more strongly detect the “rigid type I β-turn,” which is stabilized by the expansion of the glutamine repeat.

**Structural Effects of Inserted Glutamine Repeat on Other Regions of Mb—**Although it is most plausible that the β-strand of polyglutamine induces the formation of aggregates by intermolecular interactions, we cannot deny that expanded polyglu-
tamine seriously affects conformation in other regions of the protein, which facilitates the formation of aggregates. In the present study, the features of the \(^1\)H NMR spectra were not affected by the expansion of the glutamine repeat. Since \(^1\)H NMR spectra are amenable to subtle directional changes in the heme group and several heme environmental residues (22, 23), we concluded that structural changes in the protein interior, heme, and heme environment upon expansion of the glutamine repeat would be rather small. Further structural information was obtained by fluorescence spectroscopy. The similar redshift of the emission maximum of Gln\(_{35}\) and Gln\(_{50}\) Mbs to that of wild-type Mb at pH 4.2 suggested the molten globule-like state of the Mb mutants (19, 24). Such redshift of the emission maximum of the mutant Mbs was also encountered for the carbonic anhydrase in the molten globule state (25). In addition, the negative peak at 1661 cm\(^{-1}\) in the difference IR spectra suggests that some of the \(\alpha\)-helices are un wound in the mutants. Therefore, the mutant Mbs would have the intact protein core and the fluctuating protein surface as is observed for molten globules (18, 19).

Formation Mechanism of Aggregates in Proteins Containing an Expanded Polyglutamine—The surface unfolding upon expansion of glutamine repeats corresponded well with the protein stability experiment. Fig. 6C shows that the overall structures of Gln\(_{35}\) and Gln\(_{50}\) mutant Mbs were seriously destabilized, whereas the stability of the protein core was not affected by the expansion of polyglutamine. In other words, the severe destabilization of the overall structure in the Gln\(_{35}\) and Gln\(_{50}\) mutants is attributed to destabilization of protein surface.

On the basis of our results, we propose the following formation mechanism of aggregates in the proteins with expanded polyglutamine. The antiparallel \(\beta\)-pleated sheet of polyglutamine in a nonpathological length is evolutionarily stabilized in the protein and is not exposed to solvent. Once the glutamine repeat is expanded to a pathological length, the end of the \(\beta\)-pleated sheet of the expanded polyglutamine breaks through the protein surface, causing its destabilization and partial unfolding of the protein surface. This would render the exposed polyglutamine more fluctuating and accessible to another molecule. The expanded polyglutamine exposed on the protein surface readily forms intermolecular antiparallel \(\beta\)-sheets, and accumulation of the polyglutamine \(\beta\)-strands results in aggregates. Such correlation of tendency to form aggregates with destabilization of a native state has been recently reported for various mutants of a globular protein, acylphosphatase (26). This scheme of aggregate formation can be applied to the processing model in which a full-length protein is cleaved before formation of aggregate because an expanded polyglutamine destabilizes the processed smaller fragment more severely than its native full-length molecule.

In the present study, we have established a molecular model for polyglutamine diseases using sperm whale myoglobin. This is the first successful isolation and structural characterization of the protein in which various lengths of polyglutamine including a pathological repeat number were inserted. The mutant Mbs in this study would be applied for further studies such as the screening of small molecules that inhibit the formation of polyglutamine aggregates.

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