Oligopeptide-Mediated Stabilization of the α-Helix of a Prion Protein Peptide

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The helix-stabilizing effect of a small peptide on the peptide dissected from the prion protein is described. Generally, the small peptide should be a pentapeptide, αxyzβ. The xyz should be composed of hydrophobic amino acids that are found in the prion protein and are interposed by relevant basic or acidic amino acids of α and β so as to form intermolecular salt bridges with the corresponding acidic/basic amino acids in the prion protein. The pentapeptide αxyzβ could be a therapautic agent for the prion disease, because the generation of an immune response and the sensitivity of a peptide to proteolytic degradation is considered to be minimized by shortening the peptide length.

A common feature related to the pathogenic mechanisms of neurodegenerative disorders, which include Alzheimer's disease, amyotrophic lateral sclerosis, ataxic syndromes, Huntington's disease, Parkinson's disease, prion disease, and so on, is an abnormal protein deposition in brain tissues; the protein is characterized by the genetic traits of each disease.[1,2] These specific proteins are amyloidogenic and improperly fold or aggregate to produce toxic amyloid fibrils. The amyloid fibrils are considered to be produced by the conformational changes from the α-helix to β-sheet rich structures.[3] Thus, although there may be various therapautic strategies,[4] stabilizing the α-helical conformation of the specific protein that inhibits its conversion into β-sheets appears to be the most fundamental therapeutic strategy.[3,5] Stabilizing the native fold of an amyloidogenic protein by a small molecule is also useful for preventing the conformational changes.[6]

Previously, we found that a pentapeptide KIFMK, the IFM motif, which locates in the III−IV linker that connects between domains III and IV of the sodium channel α-subunit, stabilizes the α-helical conformation of the peptide, MP-1A.[7] The MP-1A includes the sequence DIFMTEE and was dissected from the III−IV linker.[7] The affinity and the binding specificity appear to originate from both the mutual hydrophobic interactions of the IFM motifs and the electrostatic interactions (salt bridges) between the two Lys residues in KIFMK and the Asp and Glu in MP-1A.[8] The helix-stabilizing effect is attributable to the intermolecular salt bridges.[8] The stability of the attainable α-helix is comparable to that of a single intramolecular salt bridge.[8]

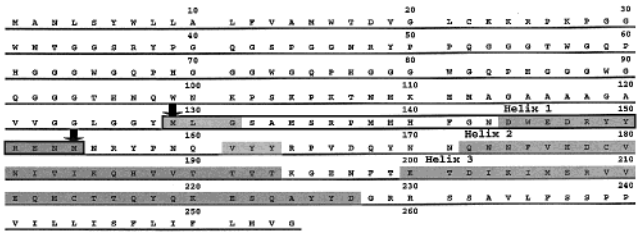

Figure 1. The amino acid sequence of the Syrian hamster prion protein.[9]

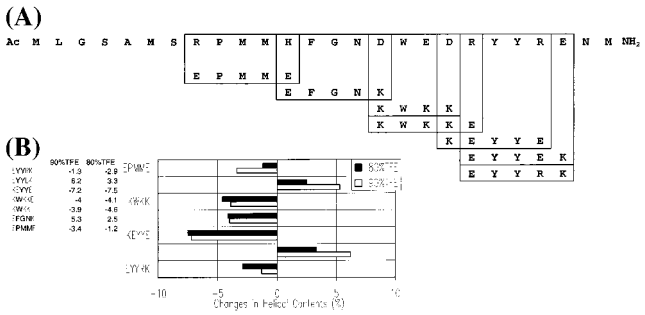

Figure 2. (A) Schematic representation of the oligopeptides chosen in the model peptide PrP(129−154). (B) Changes in the helical contents (%) of the PrP(129−154)-oligopeptide in 80% (■) and 90% (□) TFE solutions.

In this study, a search was made for an oligopeptide of the type αxyzβ that may be able to stabilize the helical conformation of the prion protein. The amino acid sequence of the Syrian hamster prion protein is shown in Figure 1.[9]

By multidimensional NMR spectroscopy for the prion protein, rPrP(90−231), in 10% D_2O/90% H_2O at pH 5.2, Liu et al. demonstrated that there exist three α-helices for residues D144-M154 (Helix 1), Q172-T193 (Helix 2), and E200-D227 (Helix 3) and the two short antiparallel β-strands for residues M129-G131 and V161-Y163.[10] Inspection of the amino acid sequence in Figure 1 suggests that there are two regions in which the present method can be applied. One is around Helix 1 and the other around Helix 3. In the former, there are many candidates that can be picked up as an αxyzβ type of fragment, because basic and/or acidic amino acids are scattered at *i*, *i*+4 intervals on the sequence around Helix 1. In the latter, for the segments E196-E200 and E207-E211, KNFTK and KRVVK, respectively, can be candidates.

Presently, we investigated the former region (Helix 1), and synthesized a model peptide PrP(129−154) consisting of M129-M154 (Figure 1); its N-terminal was acetylated and the C-terminal was amidated so as to avoid trivial electrostatic interactions at the ends. Figure 2A schematically shows the locations of the αxyzβ type of fragments selected by picking up the basic or acidic amino acids at the *i*, *i*+4 intervals. The oligopeptides were determined by reversing the acidic and basic amino acids with each other so as to form intermolecular salt bridges; Lys and Glu were employed as the basic and acidic amino acids, respectively. In a case where the "*xyz*" part includes an acidic or basic amino acid, the kind of amino acid at *x*, *y*, or *z* was also reversed to increase the affinity, except for the segment R148-E152, for which we tried both cases, i.e., EYYEK and EYYRK. This is because if the "*z*" part of Glu in EYYEK interacts with R151, the C-terminal side of the positive charge

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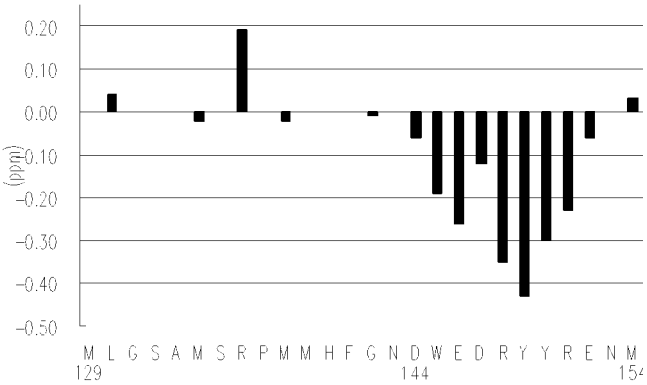

Figure 3. The Hα chemical shift differences between the experimental shifts for PrP(129−154) and the random coil shifts.

of R151 is neutralized; this situation is unfavorable for the charge-dipole interaction that stabilizes the helix.[11] The N-termini were acetylated and the C-termini were amidated so as to avoid trivial electrostatic interactions at the ends. In addition, we also tried a tetrapeptide, KWKK, for the segment D144-D147.

On the basis of sequential assignment procedures for the COSY, TOCSY, and NOESY data in the two-dimensional ^{1}H NMR spectra,[12] all the ^{1}H NMR resonances of PrP(129−154) in 90% trifluoroethanol (TFE)-d_2−10% H_2O solution (3 mM, pH 7.0, 300 K) were assigned. In accordance with Wishart et al.,[13] the relative α-proton chemical shifts to the random coil values are summarized in Figure 3. Inspection of the graph clearly shows that D144-E152 assumes a helical conformation, agreeing well with Liu et al.'s Helix 1 region, despite the difference in the solvent system.[10]

The CD spectrum of PrP(129−154) in 80% TFE−20% H_2O and 90% TFE−10% H_2O solutions (30 μM, pH 7.0, 300 K) showed double negative maxima at 206 ($\pi-\pi_{\|}$* transitions) and 222 nm (n−π* transitions) and a strong positive maximum at 190 nm ($\pi-\pi_{\perp}$* transitions), indicating that the overall secondary structure of PrP(129−154) is an α-helix.[14] The helicity estimated from the negative maximum at 222 nm was 31% at 90% TFE.[15] From the changes in ellipticity at 222 nm in the observed CD spectra of PrP(129−154) as a result of adding various kinds of oligopeptides (30 μM) to the PrP(129−154) solution (30 μM, pH 7.0, 300 K), we estimated the changes in the helical contents of the PrP(129−154)-oligopeptide solutions; the control ellipticity data were determined by summing the CD spectrum of PrP-(129−154) and that of the oligopeptide using a computer and dividing by the total residue number. This estimation method of the changes in the helical content involves those from both the PrP(129−154) and the oligopeptide. However, the change in the helicity due to the oligopeptide may be small, if any, as compared to that of PrP(129−154), because the oligopeptide is much smaller than PrP(129−154) and will contribute little to the mean residue ellipticity at 222 nm. In Figure 2B, we graphically summarized the changes in the helical contents as a percent. Evidently, EFGNK and EYYEK increased the helicity, while all the other oligopeptides decreased helical content. Unrelated control peptides tried (EAAAK, EYYEE, EEYYE, KIFMK) had no effect on the helical content except KIFMK; the KIFMK decreased helical content by 4.2% at 90% TFE. Moreover, EFGNK and EYYEK had no effect on the structure of PrP(129−154) in aqueous solution, because PrP(129−154) was assuming a random-coil conformation. Since a salt bridge is the electrostatic interaction, it is expected that if we increase the solvent hydrophobicity, the salt bridge would be strengthened. This was indeed true. As shown in Figure 2B, the changes in the helicity by EFGNK and EYYEK were increased by about twice with increasing solvent hydrophobicity. No appreciable [PrP(129−154)]/[oligopeptide] molar ratio dependences were seen for these changes in the helical contents. The average values were 5.4 ± 0.3% and 6.4 ± 0.3% respectively for EFGNK and EYYEK between 1:1 and 1:4 molar ratios. Interestingly, KEYYE, which is a reversed amino acid sequence of EYYEK, significantly destabilized the helical structure of PrP(129−154). This result indicates that the oligopeptides and PrP(129−154) are specifically interacting with each others sequence. Although it is not certain whether the chosen oligopeptide is interacting exactly with the corresponding segment in the PrP(129−154), one of the most serious concerns was whether the oligopeptides can really stabilize the helical structural part of the PrP(129−154). This is because in PrP(129−154), there is a possibility that its α-helical structure has already been stabilized by the following intrahelical salt bridges at the *i*, *i*+4 intervals,[16] i.e., H140, D144, R148, and E152; these alterations in basic and acidic amino acids at the *i*, *i*+4 intervals continue further to R156. In this case, every oligopeptide would destabilize the α-helical structure. However, the present data clearly alleviated this concern, because EFGNK and EYYEK increased the helicity. Recently, Soto et al. have shown that ^{i}PrP13 (DAPAAPAG-PAVPV), a 13 residue peptide that involves the amino acid sequence AAAAGAVV in the prion protein (A115-V122; Figure 1), proline residues to block β-sheet formation, and a charged residue (Asp) at the end to increase solubility, reverses the prion protein conformational changes implicated in the pathogenesis of spongiform encephalopathies.[17] The ^{i}PrP13 played a role as a β-sheet breaker, whereas the present αxyzβ type of pentapeptide acted as an α-helix stabilizer.

In conclusion, we demonstrated that the αxyzβ type of pentapeptide, which involves the amino acid sequence of "*xyz*" in the prion protein and which is interposed by the appropriate acidic or basic amino acids (α,β) to form intermolecular salt bridges, could be a helix-stabilizing agent for the protein. Further studies to apply the present method for a model peptide around Helix 3 in the prion protein and for another amyloidogenic protein are now in progress.

Supporting Information Available: ^{1}H NMR and CD data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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