Interaction between Human Prion Protein and Amyloid-β (Aβ) Oligomers

ROLE OF N-TERMINAL RESIDUES

Received for publication, May 17, 2010, and in revised form, June 18, 2010. Published, JBC Papers in Press, June 24, 2010, DOI 10.1074/jbc.M110.145516

Shuguí Chen‡, Satya P. Yadav§, and Witold K. Surewicz‡1

From the ‡Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106 and the §Molecular Biotechnology Core Laboratory, the Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Soluble oligomers of Aβ42 peptide are believed to play a major role in the pathogenesis of Alzheimer disease (AD). It was recently found that at least some of the neurotoxic effects of these oligomers may be mediated by specific binding to the prion protein, PrPC, on the cell surface (Laureń, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2009) Nature 457, 1128–1132). Here we characterized the interaction between synthetic Aβ42 oligomers and the recombinant human prion protein (PrP) using two biophysical techniques: site-directed spin labeling and surface plasmon resonance. Our data indicate that this binding is highly specific for a particular conformation adopted by the peptide in soluble oligomeric species. The binding appears to be essentially identical for the Met129 and Val129 polymorphic forms of human PrP, suggesting that the role of PrP codon 129 polymorphism as a risk factor in AD is due to factors unrelated to the interaction with Aβ oligomers. It was also found that in addition to the previously identified ~95–110 segment, the second region of critical importance for the interaction with Aβ42 oligomers is a cluster of basic residues at the extreme N terminus of PrP (residues 23–27). The deletion of any of these segments results in a major loss of the binding function, indicating that these two regions likely act in concert to provide a high affinity binding site for Aβ42 oligomers. This insight may help explain the interplay between the postulated protective and pathogenic roles of PrP in AD and may contribute to the development of novel therapeutic strategies as well.

Although the etiology of AD remains poorly understood, the leading hypothesis is that the major causative agent is the aggregated form of Aβ (4, 7). Although in the past much attention has focused on mature β-sheet-rich amyloid fibrils, more recent evidence points to a critical role of smaller, soluble Aβ oligomers (8–13). In contrast to poor quantitative correlation between the burden of insoluble fibrillar amyloid plaques and the degree of dementia, the severity of AD appears to correlate well with the concentration of soluble Aβ oligomers (9–11). Furthermore, these soluble oligomers have been shown to be potent neurotoxins in vitro and in vivo; among other effects, they were reported to inhibit hippocampal long term potentiation, a widely used electrophysiological measure of synaptic plasticity related to learning and memory, and cause impairment of long term memory in rats (9–11, 14–16). However, despite rapid progress in the field, the biochemical and biological mechanisms by which Aβ oligomers interact with synapses and lead to their functional impairment remain largely unknown.

An important recent development was the identification of cellular prion protein, PrPC, as a high affinity binding site for Aβ oligomers (17). Human PrPC is an abundantly expressed 209-residue glycoprotein, which largely localizes to cholesterol-rich lipid rafts on the outer surface of the cell membrane via a C-terminal glycosyldiylinositol anchor (18). Although the protein is best known for its role, in a misfolded state, as a pathogenic agent in prion diseases (18–21), it appears to have a range of normal physiological functions (22–24), playing a role in copper homeostasis, cellular signaling, neuroprotection, cell adhesion, and synaptogenesis. Using an unbiased proteome-wide screen, Lauren et al. (17) recently found that cell surface PrPC acts as a highly specific binding site for synthetic Aβ42 oligomers. Importantly, the authors also demonstrated that these soluble Aβ42 oligomers inhibit long term potentiation in hippocampal slices from wild-type mice but not in those from the PrPC–null mice. Thus, these results strongly suggest that PrPC may act as a long sought specific receptor that mediates at least some of the toxic effects of Aβ oligomers. Apart from opening new avenues in AD research, identification of such a receptor provides a potentially valuable target for pharmacological intervention. Development of any therapeutic strategies targeting Aβ oligomer–PrPC interaction would require detailed knowledge of the molecular aspects of this interaction. Here we used surface plasmon resonance (SPR) and site-directed spin labeling to characterize PrP–Aβ42 binding at the biophysical...
Interaction between Prion Protein and Aβ Oligomers

level and to identify specific segments of the prion protein that are involved in this interaction. Our data demonstrate that in addition to the previously postulated ~95–110 region, the cluster of basic residues within the N-terminal 23–27 segment of PrP is critically important for effective binding to Aβ42 oligomers.

EXPERIMENTAL PROCEDURES

DNA Constructs and Protein Purification—The plasmid encoding human prion protein (huPrP) with an N-terminal linker containing a His6 tail and a thrombin cleavage site was previously described (25). The deletion variants and Cys mutants were generated by site-directed mutagenesis using appropriate primers and the QuikChange kit (Stratagene), and the sequences were verified by DNA sequencing. Proteins were expressed and purified as described previously (25, 26). Spin labeling with the [1-oxo-2,2,5,5-tetramethyl-pyrroline-3-methyl]-methanethiosulfonate label and final purification of labeled proteins were performed as described previously (27). Protein concentration was determined by absorbance at 276 nm using extinction coefficients appropriate for each deletion variant.

Preparation of Aβ42 Oligomers and Fibrils—Aβ42 peptide was purchased from American Peptide Co. (Sunnyvale, CA) as lyophilized powder. The peptide was dissolved in hexafluoro-2-propanol, aliquoted, and stored (after solvent evaporation) at −80 °C. Immediately before use, an aliquot was dissolved in a freshly prepared 10 mM NaOH solution and disaggregated as described previously (28). The basic Aβ42 stock solution was then combined with a sodium phosphate buffer (final pH 7.4) precooled to 4 °C, and this solution was used immediately as a preparation of Aβ42 monomer. Aβ42 oligomers were generated by incubating the solution of monomeric Aβ42 (100 μM) at room temperature for 24 h (29). Aβ42 fibrils were generated by incubating the solution of the peptide in phosphate buffer (pH 7.4) for ~6 days at 37 °C or under acidic conditions (pH 2) for 24 h (30). The morphology of oligomers and fibrils was analyzed by atomic force microscopy as described previously (31).

Surface Plasmon Resonance Experiments—Interaction between human PrP and Aβ42 oligomers was probed by SPR using a Biacore 3000 instrument. Prion protein was diluted to 1 μM in 10 mM sodium acetate, pH 4.5, and immobilized on a CM5 sensor chip using the amine coupling chemistry. After coupling, the chip was deactivated with 1 M ethanolamine hydrochloride, pH 8.5. A reference channel of the chip was subjected to an identical chemical processing in the absence of the prion protein. Aβ42 monomer, oligomers, or fibrils were injected in a running buffer (150 mM NaCl, 0.005% surfactant P20, 10 mM HEPES, pH 7.4) at a flow rate of 10 μl/min followed by the injection of the same buffer in the absence of Aβ42 peptide. Typically, association and dissociation curves were monitored for 5 min each. Regeneration of the surfaces was achieved by the injection of 25 mM NaOH at the flow rate of 100 μl/min.

Electron Paramagnetic Resonance Spectroscopy and Analysis of Binding Data—Samples for EPR spectroscopy were prepared by incubating spin-labeled huPrP (2 μM) for 1 h with different forms of Aβ42 at varying concentrations. Samples were then transferred into glass capillaries, and EPR spectra were measured at room temperature using a Bruker EMX spectrometer equipped with high sensitivity probe head as described previously (27).

For construction of binding isotherms, spectra for huPrP/ Aβ42 oligomer mixtures were normalized to the same number of spins, and the amplitude of the low field sharp feature (labeled a in Fig. 5A) was measured for each spectrum. Because this amplitude is proportional to the population of free protein, the fraction of bound huPrP in the presence of different amounts of Aβ42 oligomers, ω, was calculated as ω = (A0 − A)/(A0 − Amax), where A0 is the amplitude of low field sharp feature for free huPrP, and A and Amax represent this amplitude at a given concentration of Aβ42 oligomers and under saturating conditions, respectively. To determine the apparent equilibrium binding parameters, we used a model assuming that each huPrP molecule, P, interacts with a binding site on Aβ42 oligomer, O, according to the equilibrium

\[ P + O \rightleftharpoons PO \]  

(Eq. 1)

It is further assumed that each binding site consists of n Aβ monomers. Therefore, \[ [PO] = [Aβ]_T/n\], where \[ [P] \] is the total concentration of binding sites and \[ [Aβ]_T \] is the total concentration of Aβ42. Because \[ [P]_T = [P] + [PO] \], where \[ [P]_T, [P] \] and [PO] are total, free, and bound huPrP concentrations, respectively, the dissociation constant for the equilibrium (Equation 1) can be expressed as

\[ K_d = \frac{([P]_T - [PO])([Aβ]_T/n - [PO])}{[PO]} \]  

(Eq. 2)

Given that \[ [PO] = \theta[P]_T \], where \( \theta \) is the fraction of bound huPrP as determined from EPR spectra, Equation 2 can be rearranged as

\[ \theta = 0.5 \times \left[ 1 + \frac{[Aβ]_T}{[P]_T \times n} \times \frac{K_d}{[P]_T} \right] - \left( \frac{[Aβ]_T}{[P]_T \times n} \right)^2 \]  

(Eq. 3)

Nonlinear regression fitting of experimental data according to Equation 3 (using the Origin software) was used to determine the binding parameters \( K_d \) and \( n \).

RESULTS

Binding of Aβ42 Oligomers to PrP as Probed by Surface Plasmon Resonance—The preparations of Aβ42 oligomers used in the present study were examined by atomic force microscopy; they appear as spherical particles with a typical diameter (height in atomic force microscopy images) of 2–5 nm, with no fibrillar aggregates present (Fig. 1, A and B). These preparations appear morphologically similar to those used in previous studies on Aβ42 interaction with the prion protein (17, 32). Fig. 1 also shows atomic force microscopy images of mature Aβ42 fibrils (panel C) as well as fibrils fragmented by sonication (panel D).

The binding interaction between recombinant full-length huPrP and different forms of Aβ42 was investigated using SPR. The protein was immobilized on the surface of a CM5 sensor.
Interaction between Prion Protein and Aβ Oligomers

A, oligomers at low magnification; B, the same oligomers at higher magnification; C, amyloid fibrils; D, amyloid fibrils fragmented by sonication. The scale bars correspond to 1 μm.

These assemblies, long fibrils were fragmented by sonication. Also, in this case, no binding to huPrP could be detected by SPR (Fig. 2B).

In many cases, analysis of SPR data allows calculation of binding constants from the ratio of rate constants for the association and dissociation reactions (33). However, consistent with the previous observation (32), the dissociation parts of the sensorgrams for the huPrP-Aβ42 oligomer interaction were highly unusual in that they were almost horizontal, indicating very slow effective dissociation kinetics. This could be due to a number of factors, including secondary binding events that sometimes occur in SPR experiment and/or the obvious potential for multivalent binding between surface-immobilized PrP and large, heterogeneous in size oligomers consisting of many Aβ42 molecules. In any case, sensorgrams such as those shown in Fig. 2A do not allow reliable determination of dissociation rates. This, combined with the lack of analytical models for binding of heterogeneous oligomeric ligands, precluded quantitative assessment of SPR data in terms of binding constants. Therefore, we kept our analysis at a qualitative level, using the response (measured in resonance units) as an empirical measure of relative binding affinity for different deletion variants of huPrP. This approach, used successfully in previous SPR studies with other systems (34, 35), provides a tool for the assessment of the role of specific huPrP regions in the interaction with Aβ42 oligomers. To this end, we expressed and purified to homogeneity a number of deletion mutants of huPrP (Fig. 3A). Each of these proteins was immobilized onto the surface of a CM5 sensor chip at a density corresponding to ~4000 resonance units (RU) (4000 ± 300, and the Aβ42 oligomers were injected at a concentration of 2 μM.

chip, and Aβ42 preparations were then injected (binding phase) followed by the injection of buffer alone (dissociation phase). Typical sensorgrams at several concentrations of Aβ42 oligomers are shown in Fig. 2A; they clearly demonstrate concentration-dependent binding of these oligomers to immobilized huPrP. Essentially identical results were obtained using Met129 and Val129 huPrP (data for Val129 not shown for brevity), indicating that the Met/Val polymorphism at residue 129 does not affect prion protein interaction with Aβ42 oligomers. In sharp contrast to the oligomers, no binding was detected for Aβ42 monomers or mature amyloid fibrils (Fig. 2B). To test whether the striking differences between the oligomers and fibrillar aggregates are not just due to the difference in size of
Interaction between Prion Protein and Aβ Oligomers

entire N-terminal region 23–89 was removed (Δ23–89 huPrP), finding that this deletion results in essentially complete loss of binding capacity to Aβ42 oligomers (as indicated by the lack of any response in the sensogram; Fig. 3B). Building on this observation, we then tested variants with partial deletions within the 23–89 region, Δ23–50 and Δ51–91. Although the deletion of residues 51–91 did not have any measurable effect, the removal of N-terminal residues 23–50 resulted in approximately an 8-fold reduction of the response in the sensogram, indicating greatly reduced binding of Aβ42 oligomers (Fig. 3B).

A similar impairment of Aβ42 binding was observed for the deletion variant Δ23–27 (Fig. 2B), indicating that the N-terminal cluster of basic residues 23KKRPK27 is critical for the interactions between prion protein and Aβ42 oligomers.

To test the role of other regions, three additional deletion mutants were generated, Δ92–110 huPrP, Δ101–110 huPrP, and Δ111–125 huPrP. SPR data show that the removal of residues 92–110 results in a major loss of binding capacity (Fig. 3). The cluster of basic residues within the 101–110 segment appears to be only partly responsible for this effect as only partial loss of binding was observed for Δ101–110 huPrP. Finally, the sensogram for the Δ111–125 variant was essentially identical to that for wild-type huPrP (Fig. 3C), indicating that the hydrophobic region 112–122 is not important for huPrP interaction with Aβ42 oligomers.

Site-directed Spin Labeling Experiments—The interaction between huPrP and Aβ42 was further probed by site-directed spin labeling coupled with EPR spectroscopy (36, 37). In this approach, Gly at position 30 was replaced with Cys, and the latter residue was derivatized with the nitroxide label. The EPR spectrum of spin-labeled huPrP labeled at residue 30 (30R huPrP) in solution shows three relatively sharp lines (Fig. 4A). Such a spectrum is characteristic of a highly mobile nitroxide label (36, 37), consistent with NMR data showing that the N-terminal part of huPrP is largely unstructured and flexible (38). If, as indicated by SPR data, the N-terminal part of PrP is involved in the interaction with Aβ42 oligomers, one would expect this interaction to result in a significant immobilization of the nitroxide label attached to residues within this region. Indeed, the EPR spectrum of 30R huPrP in the presence of Aβ42 oligomers (Aβ42 monomer to huPrP of 50:1) is dramatically different from that of the free protein, showing broad features characteristic of highly immobilized nitroxide (Fig. 4B). In contrast, no immobilization of the label was observed in the presence of Aβ42 monomer, mature amyloid fibrils, or fibrils fragmented by sonication. Thus, EPR data provide further evidence that huPrP interacts selectively with Aβ42 oligomers and has no apparent affinity for the monomeric peptide, amyloid fibrils or amyloid assemblies fragmented by sonication (Fig. 4, C–E).

As shown previously for other systems (39), distinct spectral properties of free and bound 30R huPrP provide a tool for quantitative analysis of equilibrium binding between prion protein and Aβ42 oligomers. To this end, 30R huPrP (2 μM) was titrated with increasing amounts of Aβ42 oligomers, and EPR spectra were recorded for each mixture. As shown in Fig. 5A, upon increasing the concentration of Aβ42, there was a decrease in the amplitude of low field sharp feature (labeled a), the intensity of which is a measure of the population of free huPrP. This was accompanied by an increase in the intensity of the immobilized component characteristic of the bound species (labeled b). For each huPrP/Aβ42 ratio, the molar fraction of free and bound 30R huPrP was determined from the intensity of the low field sharp feature (see “Experimental Procedures”), and these data were used to generate a binding isotherm (Fig. 5B). Fitting this isotherm to a simple equilibrium binding model (see “Experimental Procedures”) yields an apparent dissociation constant, K_d, of 71 nM and the number of Aβ42 monomers per binding site of 21.

Next we used the site-directed spin labeling method to probe the effect of specific huPrP deletions on binding to Aβ42 oligomers. As shown in Fig. 6A, even at very high concentrations of Aβ42 oligomers, EPR spectra for 30R huPrP with deletions of residues 92–110 or 23–27 show only a very weak immobilized component. Because it is difficult to maintain Aβ42 in the oligomeric (nonfibrillar) state at peptide concentrations above ~100 μM, we could not construct binding isotherms for these deletion variants. However, the much lower intensity of the immobilized component in EPR spectra for Δ92–110 huPrP and Δ23–27 huPrP as compared with wild-type protein clearly demonstrates that both deletions greatly impair huPrP binding to Aβ42 oligomers. In contrast, the spectra for Δ111–125 30R huPrP in the pres-
ence of Aβ42 oligomers were very similar to those for wild-type protein, demonstrating that residues 111–125 are of little importance for Aβ42 oligomer binding.

To assess the effect of the deletion of the entire 23–89 segment, we prepared a huPrP variant with the nitroxide at residue 113 (113R huPrP). As expected, the EPR spectrum of free 113R huPrP displays three relatively sharp lines characteristic of highly mobile nitroxide, whereas in the presence of Aβ42 oligomers, the spectrum shows features characteristic of an immobilized nitroxide (Fig. 6B), indicating formation of a complex. In contrast, no immobilized component could be detected in the spectrum of the 23–89 113R huPrP mixture with Aβ42 oligomers, confirming the SPR-based observation that the deletion of residues 23–89 completely abolishes huPrP binding to Aβ42 oligomers. In each case, the concentration of the prion protein and Aβ42 was 2 and 100 μM, respectively. For visualization purposes, spectra are scaled to the same vertical size.

DISCUSSION

A number of recent observations point to an intriguing link between the cellular form of prion protein, PrPC, and AD (see Ref. 40 for review). Early studies in this regard focused on genetic analysis, finding that the gene encoding human prion protein (PRNP) is a potential AD susceptibility gene (41) and that Met/Val polymorphism at codon 129 in PRNP may be a risk factor for AD in certain populations (42, 43). More recently, it was found that PrPC inhibits cleavage of APP by β-secretase BACE1, thereby decreasing production of Aβ peptides (44). This effect, suggestive that PrPC may have a protective role against AD, appears to be modulated by PRNP polymorphism at codon 129. The most recent twist is the finding that PrPC may act as a high affinity receptor for Aβ42 oligomers (17). Binding to this receptor was found to mediate Aβ42 oligomer-induced inhibition of long term potentiation in hippocampal slices, as indicated by the lack of such inhibition in slices derived from mice in which the Prp gene was knocked out (17). The latter observation strongly suggests that PrPC mediates at least some of the toxic effects of Aβ oligomers and, thus, plays an important role in AD pathogenesis, although the clinical relevance of these findings is yet to be demonstrated (32).
Interaction between Prion Protein and Aβ Oligomers

protective function of PrP\textsuperscript{C} as a modulator of APP processing and its pathogenic role as a modulator of Aβ toxicity are not necessarily mutually exclusive; rather, they suggest a possibility of an intriguing feedback loop between these effects (40).

Because the finding regarding the role of PrP\textsuperscript{C} as a mediator of Aβ oligomer toxicity has potentially major implications for AD research, it is important to understand the mechanism of the interaction between these two proteins at the molecular level. This can be best studied by biophysical methods using purified prion protein, the approach that avoids interference of other potential Aβ binding partners present in the cellular environment. Here we characterized the interaction between recombinant human PrP prion protein and Aβ\textsubscript{42} using two complementary biophysical methods, SPR and the site-directed spin labeling. Consistent with previous observations in COS-7 cells and hippocampal neurons (17), both biophysical techniques consistently demonstrate that huPrP interacts strongly with soluble Aβ\textsubscript{42} oligomers, whereas there is no detectable interaction with the monomeric form of the peptide or mature amyloid fibrils. Because previous data indicate that cytotoxic effects of fibrillar aggregates may depend on fibril length (45), we also examined the effect of fibril fragmentation on Aβ\textsubscript{42} binding. However, akin to long fibrils, no interaction was detected for fibrils that were fragmented by sonication. Thus, selective binding of huPrP observed for soluble oligomers is not related to the smaller size of these assemblies but reflects the strong specificity of the interaction for a particular conformation of Aβ\textsubscript{42} in soluble oligomeric species. The apparent dissociation constant for this interaction is \(\sim 70 \text{ nm} \), a value remarkably close to that estimated by Lauren et al. (17) for binding of similar oligomers to PrP\textsuperscript{C}-expressing COS-7 cells. Furthermore, formal analysis of our data indicates that the oligomer binding site consists of \(\sim 20\) Aβ\textsubscript{42} monomers. Given the unavoidable heterogeneity of our (and any other) preparations of synthetic Aβ\textsubscript{42} oligomers, this should be considered an average value, possibly corresponding to the average size of oligomeric species.

The main focus of the present study is on precise mapping of specific huPrP regions responsible for the interaction with Aβ\textsubscript{42} oligomers. Based on limited data, it was previously postulated that the 95–110 region is a principal site for Aβ\textsubscript{42} binding (17). However, to our surprise, we found that the deletion of the 23–90 segment results in a complete loss of huPrP binding to Aβ\textsubscript{42} oligomers, clearly indicating that residues within this N-terminal region play a major role in the interaction. These critical residues were identified as a cluster of basic amino acids KKRPK\textsuperscript{27} at the very N terminus of huPrP (Fig. 3A). The second determinant of binding to Aβ\textsubscript{42} oligomers appears to be the region \(\sim 92–110\), consistent with the previous finding regarding the role of residues 95–110 (17). The highly charged, lysine-rich cluster within this \(92–110\) region is only a part of this determinant, as indicated by a partial loss of binding observed for Δ101–110 huPrP. Our present data clearly demonstrate that both the N-terminal residues 23–27 as well as the \(92–110\) region are critically important for PrP interactions with Aβ\textsubscript{42} oligomers as the deletion of any of these regions results in a major loss of the binding function. Although this strongly suggests that these two segments act in concert to provide high affinity binding sites for Aβ\textsubscript{42} oligomers, one cannot formally rule out the possibility that for one of them, the effect on interaction could be via influencing conformation of the whole protein rather than by direct binding to the Aβ oligomer. The latter scenario is, however, less likely because both the 23–27 and the \(92–110\) regions are located within the part of the prion protein that is highly flexible and natively unstructured (38). Furthermore, NMR studies clearly demonstrate that the deletion of this entire unstructured region in PrP (up to residue \(\sim 120\)) has no effect on the conformation of the folded C-terminal domain (38). In contrast to the critical role of specific segments within the N-terminal part of PrP, the folded C-terminal domain does not appear to be directly involved in the interaction with Aβ\textsubscript{42} oligomers. The region around residue 129 is also not involved, as indicated by very similar binding observed for the Met\textsuperscript{129} and Val\textsuperscript{129} polymorphic forms of huPrP. Thus, the role of codon 129 polymorphism as a risk factor in AD must be due to factors unrelated to PrP interaction with Aβ\textsubscript{42} oligomers.

The same polybasic region at the extreme N terminus of PrP that we identified as a crucial determinant of binding to Aβ\textsubscript{42} oligomers has been previously found to be critical for the interaction of prion protein with β-secretase and the resulting inhibition of its APP-cleaving activity (44). The observation that the same region of PrP mediates the potential protective effect of the protein in AD (inhibition of β-secretase cleavage) and its toxic role (Aβ\textsubscript{42} oligomer-induced long term potentiation blockade) is highly intriguing, suggesting that there may be a subtle balance between these effects that is controlled by additional cellular cofactors. One group of likely cofactors is glycosaminoglycans, which compete for the same polybasic binding site at the PrP N terminus (46, 47) and appear to be required for the inhibitory action of PrP\textsuperscript{C} on APP cleavage by β-secretase (44). Apart from providing insight into the mechanism by which the prion protein could modulate the pathogenic process in AD, the identification of specific PrP regions that are crucial for the interaction with Aβ\textsubscript{42} oligomers may also contribute to the development of therapeutic strategies that target this interaction.

Acknowledgment—We thank Dr. Krystyna Surewicz for providing Val\textsuperscript{129} huPrP.

REFERENCES