Formation of Soluble Oligomers and Amyloid Fibrils with Physical Properties of the Scrapie Isoform of the Prion Protein from the C-terminal Domain of Recombinant Murine Prion Protein mPrP-(121–231)*

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Prion diseases are fatal neurodegenerative disorders associated with conformational conversion of the cellular prion protein, PrPC, into a misfolded, protease-resistant form, PrPSc. Here we show, for the first time, the oligomerization and fibrilization of the C-terminal domain of murine PrP, mPrP-(121–231), which lacks the entire unstructured N-terminal domain of the protein. In particular, the construct we used lacks amino acid residues 106–120 from the so-called amyloidogenic core of PrP (residues 106–126). Amyloid formation was accompanied by acquisition of resistance to protease K digestion. Aggregation of mPrP-(121–231) was investigated using a combination of biophysical and biochemical techniques at pH 4.0, 5.5, and 7.0 and at 37 and 65 °C. Under partially denaturing conditions (65 °C), aggregates of different morphologies ranging from soluble oligomers to mature amyloid fibrils of mPrP-(121–231) were formed. Transmission electron microscopy analysis showed that roughly spherical aggregates were readily formed when the protein was incubated at pH 5.5 and 65 °C for 1 h, whereas prolonged incubation led to the formation of mature amyloid fibrils. Samples incubated at 65 °C at pH 4.0 or 7.0 presented an initial mixture of oligomers and protofibrils or fibrils. Electrophoretic analysis of samples incubated at 65 °C revealed formation of sodium dodecyl sulfate-resistant oligomers (dimers, trimers, and tetramers) and higher molecular weight aggregates of mPrP-(121–231). These results demonstrate that formation of an amyloid form with physical properties of PrPSc can be achieved in the absence of the flexible N-terminal domain and, in particular, of residues 106–120 of PrP and does not require other cellular factors or a PrPSc template.

The conformational conversion of the normal cellular isoform of the prion protein, PrPC, into an abnormal pathological isoform, PrPSc, underlies a group of fatal neurodegenerative disorders known as transmissible spongiform encephalopathies or prion diseases, which includes Creutzfeldt-Jakob disease in humans, scrapie in sheep and goats, and bovine spongiform encephalopathy in cattle (1). Neuropathologically, prion diseases are characterized by neuronal loss and astroglial reaction often by spongiform degeneration of the brain and deposition of amyloid plaques (1). The unique feature of these diseases is that, in addition to sporadic and inherited forms, they may be acquired by transmission of an infectious agent. The "protein-only" hypothesis of prion propagation postulates that the abnormal isoform, PrPSc, acts as a transmissible agent of the disease and self-propagates its pathological conformation using PrPC as a substrate (1).

PrPSc is defined as an aggregated form of PrP that is largely resistant to protease K (PK) digestion under conditions in which PrPC and most other proteins are readily degraded (2). In addition to their different stabilities to proteolytic degradation, the secondary, tertiary, and quaternary structures of PrPC and PrPSc also differ (3–7). PrPC is monomeric and highly α-helical (5), whereas PrPSc adopts a multimeric arrangement and contains a large amount of β-structure and less helical structure. The transition between PrPC and PrPSc occurs by a post-translational mechanism and appears to take place without any detectable covalent modification of the protein (8). To date, however, the molecular mechanisms underlying the conformational transition between the normal and pathogenic conformers of PrP remain poorly defined. According to current models, the conformation of the prion protein fluctuates between a dominant native state, PrP0, and a series of minor conformers that self-associate in an ordered manner to produce a stable supramolecular structure, PrPSc, composed of misfolded PrP monomers (2, 9, 10). Once a stable "seed" structure is formed, additional PrP molecules can then be recruited, leading to an autocatalytic, fast formation of PrPSc (2, 10, 11).

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Amyloid aggregation plays important roles in several other human pathologies, including Alzheimer, Parkinson, and Huntington diseases. Regardless of the different amino acid sequences of the proteins or peptides from which they are formed, protein amyloids show many common physical and tinctorial properties (12). Several recent studies have shown that partially denaturing conditions are required for the in vitro formation of amyloid, which can be attributed to the necessity of destabilizing the native fold of a protein under conditions in which non-covalent interactions involving the polypeptide chain remain favorable (for reviews, see Ref. 12). In this context, use of partially denaturing in vitro conditions should be viewed as a tool to populate aggregation-prone conformations and to accelerate a process that might normally take much longer in vivo. Such studies may lead to improved understanding of the mechanisms of amyloid formation and to the development of therapeutic strategies aimed at preventing the formation of amyloid and combating amyloid diseases.

Considerable evidence now indicates that the main neurotoxic species involved in neuronal damage in Alzheimer, Parkinson, and prion diseases are soluble amyloid oligomers rather than the mature fibrils that are found in amyloid deposits in vivo (for reviews, see Refs. 13–15). This likely explains the lack of direct correlation between amyloid plaque burden and neurodegeneration observed in prion diseases (for a review, see Ref. 16) and suggests that neurotoxic soluble oligomers may play a role in prion-induced neuronal dysfunction.

The three-dimensional structures of recombinant prion proteins from a number of different organisms, including mouse, hamster, and human PrP, have revealed that the entire N-terminal segment comprising amino acid residues 23–120 is flexibly disordered and that only the segment containing the C-terminal 111 residues, PrP-(121–231), possesses a defined three-dimensional structure (17–20). The N-terminal flexible region appears to play an important role in transmissible spongiform encephalopathy transmission and influences the formation of protease-resistant conformations of PrP (e.g. Ref. 21). The N-terminal domain also contains amino acid sequences that are generally considered important in the control of aggregation (e.g. residues 94–113; Ref. 21) and the so-called amyloidogenic core consisting of the neurotoxic peptide sequence 106–126. The C-terminal region is a well structured, self-folded domain and contains three α-helices and a two-stranded antiparallel β-sheet (19, 20).

In the present work, we describe the oligomerization and fibrillization of the C-terminal domain of the murine prion protein, mPrP-(121–231). This construct lacks the entire N-terminal flexible region and most of the amino acid residues from the 106–126 amyloidogenic core of PrP. We show that various types of aggregates, including SDS-resistant oligomers and mature amyloid fibrils, are formed upon incubation of mPrP-(121–231) under different partially denaturing conditions. Formation of oligomers was optimal at pH 5.5, and some of the species formed exhibited annular morphology. Interestingly, aggregation of mPrP-(121–231) at pH 7.0 gave rise to the formation of an amyloid aggregate that was resistant to proteinase K digestion, suggesting that it exhibits properties similar to PrPSc. Possible implications of the formation of PrP oligomers to the pathogenesis of prion diseases are discussed.

EXPERIMENTAL PROCEDURES

Materials—Thioflavin T (ThT) and proteinase K were from Sigma. Uranyl acetate was from Electron Microscopy Sciences (Fort Washington, PA). All other reagents were of the highest analytical grade commercially available.

Protein Expression and Purification—The plasmid for bacterial expression of F175W variant of murine PrP-(121–231) was a generous gift from Dr. R. Glockshuber (Eidgenössische Technische Hochschule, Switzerland). The recombinant protein was expressed in Escherichia coli strain BL21(DE3) and was purified as described previously (25). After the final step of purification, mPrP-(121–231) was dialyzed against MilliQ water and concentrated using an Amicon ultrafiltration stirred cell (Amicon, Inc., Beverly, MA), and its concentration was determined using ε280 = 28,800 M⁻¹ cm⁻¹ (22). Protein aliquots (1.0–2.0 mg/ml) were stored at −20 °C until use.

PrP Aggregation—The concentration of mPrP-(121–231) in all samples was 0.8 mg/ml (60 µM), and 0.02% NaN₃ was added to prevent bacterial growth during long incubation periods. Samples were incubated for increasing time intervals at different temperatures and pH values as described in “Results.” When buffered solutions were used, the pH of the samples was adjusted with 80 mM acetic acid-NaOH (pH 4.0), 80 mM Mes·NaOH (pH 5.5), or 80 mM Tris·HCl (pH 7.0). In experiments in which aggregation was investigated in low ionic strength conditions (unbuffered solutions), samples were prepared by addition of an aliquot from the stock mPrP solution to MilliQ water, the pH of which had been previously adjusted to the desired value with HCl. After 1 week of incubation, the pH of such unbuffered solutions changed from 5.5 and 7.0 to 6.2 and 7.4, respectively.

Turbidity and Light Scattering Measurements—Protein aggregation was monitored at 25 °C by turbidimetry (optical density at 330 nm) on an Ultraspec 2000 Pharmacia spectrophotometer (GE Healthcare) and by right-angle light scattering measurements at 500 nm on an ISS PC1 spectrofluorometer (ISS Inc., Champaign, IL). Pre-aggregated samples were diluted 10× before measurements.

ThT Fluorescence Measurements—PrP samples were incubated with an equimolar concentration of ThT for 30 min at room temperature before measurements. ThT fluorescence emission spectra (excitation at 440 nm, emission from 460 to 600 nm, 5 nm bandpasses for both excitation and emission) were measured at 25 °C on an F4500 Hitachi spectrofluorometer (Hitachi Instrument Co., Tokyo, Japan). ThT exhibits very little fluorescence in aqueous buffer, and its fluorescence is greatly enhanced upon binding to amyloid fibrils (23). The increase in fluorescence upon binding thus may be used to follow the kinetics of amyloid fibril formation. Relative ThT fluorescence intensities in different pH buffers are normalized by the values measured for control mPrP-(121–231) samples that were kept at 4 °C for 1 week at the corresponding pHs.

Intrinsic Fluorescence Measurements—Fluorescence emission spectra were measured at 25 °C on an ISS PC1 spectrofluorometer with excitation at 280 nm, emission from 300 to 420

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nm, using a Schott WG 320 filter on the emission channel and 4 nm bandpasses for excitation and emission.

Gel Electrophoresis—Samples withdrawn at different time points during the aggregation of mPrP-(121–231) were analyzed by PAGE under both native (12% acrylamide) and denaturing (18% acrylamide) conditions. For native PAGE, samples were diluted in non-denaturing loading buffer. Prior to SDS-PAGE, samples were boiled for 2 min in loading buffer containing 2% SDS and 140 mM dithiothreitol. Carbonic anhydrase (29 kDa), ovalbumin (45 kDa), albumin (66 kDa), and Na,K-ATPase (α-subunit, 100 kDa) or a prestained molecular weight marker set (Pierce) were used as standards to determine the molecular masses of PrP oligomers and higher order aggregates.

Transmission Electron Microscopy—Samples were adsorbed for 2 min onto formvar-carbon-coated grids that had been pre-treated with 0.01% aqueous pol-y-l-lysine. Samples were stained with 5 μl of 2% uranyl acetate, air-dried and observed on a 902 Zeiss electron microscope at an accelerating voltage of 80 kV.

Proteinase K Digestion—Samples of either monomeric or aggregated mPrP-(121–231) (formed upon aggregation of 0.8 mg/ml mPrP-(121–231) at pH 7.0 and 65 °C for 1 week) were 4-fold diluted and incubated for 1 h at 37 °C in 100 mM Tris-HCl buffer, pH 7.2, with proteinase K at the protease:PrP ratios indicated in “Results.” Digestion was stopped by addition of 5× concentrated SDS-PAGE sample loading buffer containing 2% SDS and 140 mM dithiothreitol. Samples were heated at 95 °C for 5 min and resolved in 18% SDS-PAGE gels.

RESULTS

Temperature and pH Effects on the Aggregation of mPrP-(121–231)—To identify experimental conditions leading to the oligomerization or fibrillization of mPrP-(121–231), we initially monitored the aggregation of the protein at both pH 5.5 and 7.0 and at 37 and 65 °C. The experiments at pH 5.5 were carried out to approximate the pH in endocytic vesicles, which have been implicated as a possible intracellular compartment in which the conformational transition of PrPc to PrPSc may take place (4, 24). On the other hand, comparison between aggregation at 37 and 65 °C was motivated by the fact that the T_m for thermal denaturation of mPrP-(121–231) is ~65 °C (25–27), which should populate a partially denatured state of the protein that might be more prone to aggregation.

Initial experiments with samples incubated at 37 °C in unbuffered media for as long as 1 week at either pH showed no significant aggregation. By contrast, aggregation was markedly enhanced at 65 °C (supplemental Fig. S1). Interestingly, whereas turbidity measurements indicated that formation of mPrP aggregates was essentially complete within a few hours at 65 °C (supplemental Fig. S1, A and B), ThT binding assays showed that substantial amounts of amyloid material were only formed after much longer incubation times (i.e. several days; supplemental Fig. S1, C and D). This indicates that the aggregates initially formed slowly converted to amyloid structures with longer incubation times.

mPrP-(121–231) has been reported to adopt an alternative β-sheet conformation below pH 5 in the presence of urea (28). This prompted us to investigate aggregation at pH 4 in the absence of chemical denaturants using buffered solutions. Initial investigation of the temperature dependence of aggregation in buffered media at pH 5.5 and 7 showed that aggregation increased slightly from 37 to 59 °C, followed by a marked increase at 65 °C (supplemental Fig. S2). Based on these results, we next carried out a detailed investigation of the aggregation of mPrP-(121–231) at both 37 and 65 °C.

Fig. 1 (A–C) shows the aggregation of mPrP-(121–231) monitored by turbidimetry. Aggregation at both pH 5.5 and 7.0 was enhanced at 65 °C, with maximal aggregation achieved in as little as 2 min. We note that incubation at 65 °C did not stimulate the aggregation of mPrP-(121–231) at pH 4.0 (A). This is in apparent contradiction with previous works that showed that low pH is more effective when used in conjunction with partial denaturation by urea (e.g. Ref. 28). It is well known, however, that different chemical (e.g. urea, guanidine) or physical denaturing agents (e.g. temperature, pressure) may lead to different denatured or partially denatured ensembles. It is likely, therefore, that the partially denatured states of mPrP stabilized by urea or by elevated temperatures are different in their residual structures and in their propensities to form amyloid aggregates. It is also possible that the partially denatured conformation of mPrP stabilized at pH 4 and 65 °C exhibits slower aggregation kinetics (as supported by ThT binding results described below).

At 37 °C incubation of mPrP-(121–231) for up to 1 week at either pH 4.0 (Fig. 1D) or pH 7.0 (Fig. 1F) did not result in fibrillization as measured by ThT binding. On the other hand, samples incubated for 1 week at pH 5.5 at 37 °C (E) exhibited ~4-fold increase in ThT binding, indicating the formation of some amyloid structure under these conditions. At 65 °C, samples incubated at pH 4.0 (D) exhibited increasing ThT binding as a function of time. At pH 7.0 and 65 °C, ThT fluorescence measurements suggested that amyloid aggregates of mPrP-(121–231) were readily formed within the first hour of incubation (Fig. 1F).

Light scattering measurements indicated that incubation of mPrP-(121–231) at pH 4.0 and 65 °C for 1 week (Fig. 1G) resulted in noticeable aggregation (compatible with ThT results). Samples incubated at pH 5.5 at 65 °C (H) exhibited increasing aggregation as a function of time as monitored by light scattering. In line with the turbidity measurements, at pH 7 light scattering measurements showed that incubation at 65 °C led to very rapid aggregation of mPrP (I).

Aggregation of mPrP was also monitored by its intrinsic fluorescence emission. The mPrP construct we have utilized exhibits a 2.5-fold increase in intrinsic fluorescence upon unfolding (29). At 37 °C, incubation of mPrP-(121–231) for up to 1 week at all pH values investigated revealed no significant changes in intrinsic fluorescence, indicating preservation of the native fold of the protein. By contrast, samples incubated at 65 °C exhibited significant fluorescence increases, indicating unfolding of the protein within the first few hours of incubation (Fig. 1, J–L). The intrinsic fluorescence intensities of mPrP-(121–231) were higher within the first few hours of incubation at 65 °C and decreased progressively over time to values approaching those of control samples. This suggests that incubation of mPrP at 65 °C leads to initial (partial) unfolding of the protein and that, upon formation of amyloid aggregates, mPrP assumes a more compact conformation again and/or becomes
part of some organized structure in which Trp-175 becomes shielded from the solvent.

**Oligomers and Higher Order mPrP Aggregates Revealed by Native and Denaturing Gel Electrophoresis**—Native PAGE showed that samples incubated at 65 °C were fully converted within the first few hours into high molecular weight aggregates that did not enter the running gel (supplemental Fig. S3), whereas samples incubated at 37 °C remained monomeric for up to 1 week of incubation.

Formation of aggregates of mPrP-(121–231) was further investigated by SDS-PAGE (Fig. 2). Samples incubated at 65 °C showed progressive accumulation of SDS-resistant oligomers (dimers, trimers, and tetramers) and higher molecular weight aggregates at all pH values investigated. Prolonged incubation at 65 °C also led to the appearance of low molecular mass bands (<13 kDa), which likely correspond to proteolysis products of mPrP-(121–231). It is interesting to note that even these lower molecular mass fragments of mPrP appeared to undergo aggregation at 65 °C, giving rise to faint bands between the monomer/dimer and dimer/trimer bands.

**Ultrastructural Analysis of mPrP Aggregates**—The morphologies of aggregates formed under different experimental conditions were examined by transmission electron microscopy (Fig. 3). Inspection of a large number of negatively stained control samples (i.e. samples kept at 4 °C for up to 1 week at different pH values) showed absence of oligomeric or polymeric structures (data not shown). Samples incubated for up to 1 week at 37 °C also showed absence of aggregated material (data not shown).

Samples incubated for as little as 1 h at pH 5.5 and 65 °C in unbuffered solution exhibited large numbers of roughly spherical aggregates with average diameter of ~12 nm (with larger particles with diameters reaching up to ~30 nm; Fig. 3A). It is also interesting to note that some of the aggregates exhibited distinct annular structures (Fig. 3A, inset), reminiscent of the morphologies exhibited by aggregates of α-synuclein, amylin, and serum amyloid A (30). Longer incubation times at pH 5.5 led to the disappearance of the spherical aggregates and to the appearance of mature amyloid fibrils with diameters of ~15–30 nm (Fig. 3B).

**FIGURE 1.** Influence of pH and temperature on the aggregation of mPrP-(121–231) in buffered solutions. The pH of the samples was adjusted with the following buffers: 80 mM acetic acid-NaOH, pH 4.0; 80 mM Mes-NaOH, pH 5.5; 80 mM Tris-HCl, pH 7.0. The samples were incubated at pH 4.0 (A, D, G, and J), pH 5.5 (B, E, H, and K) or pH 7.0 (C, F, I, and L) at 37 or 65 °C for the indicated periods of time (in hours). The concentration of mPrP-(121–231) was 0.8 mg/ml. Aggregation was monitored by optical density, light scattering, thioflavin T, or intrinsic fluorescence measurements. The insets in B and C show the initial kinetics of mPrP aggregation (times shown in minutes on the abscissa) measured by turbidimetry. Bars in the insets correspond to means ± S.D. from three determinations. For ThT fluorescence measurements (D–F), Student’s t test (single-tailed) analysis revealed that the values measured at all time points at 65 °C were significantly different (0.003 < p < 0.04) from the value of control, non-aggregated samples. Additional individual differences between values measured at different time points are indicated by * (p = 0.03) and ** (p = 0.01). The bars represent means ± S.E. of three to four independent experiments.

mPrP-(121–231) samples incubated for 1 h at 65 °C and pH 7.0 in unbuffered solution showed abundant oligomers and some short protofibrillar structures (Fig. 3C). Longer incubation times under these conditions led to a decrease in the num-
Protease Resistance of Aggregated mPrP—Proteinase K digestion has been widely used to distinguish between protease-resistant PrPSc and protease-labile PrPSc conformations of the prion protein as well as to probe differences between PrPSc strains (31, 32). Treatment of native mPrP-(121–231) with proteinase K resulted in complete degradation of the protein even at very low protease:PrP ratios (Fig. 4, upper panel). By contrast, aggregation for 1 week at 65 °C and pH 7.0 was accompanied by a significant increase in resistance to proteinase K digestion (Fig. 4, lower panel).

DISCUSSION

The construct used in the present work corresponds to the structured 111-residue C-terminal domain of the murine cellular prion protein and lacks the entire flexible N-terminal region, which has been implicated in transmissible spongiform encephalopathy transmission and in the formation of protease-resistant aggregates (21). Moreover, the segment comprising amino acid residues 106–126 in full-length PrP represents the most conserved and hydrophobic region, is known to form neurotoxic aggregates in vitro, and is generally considered to form the amyloidogenic core of the protein (33). Although recombinant mPrP-(121–231) contains only six residues from that hydrophobic region, we show that it has the ability to assemble into roughly spherical soluble aggregates, annular and linear protofibrils, and amyloid fibrils under appropriate experimental conditions in the absence of any other cellular factors. mPrP-(121–231) has been shown to form amyloid aggregates in the presence of DNA (25), but the present results show that nucleic acid is not required for amyloid aggregation of this PrP construct. Taken together, the present results support the notion that the structured C-terminal domain of PrP may play important roles in amyloid aggregation and in the formation of proteinase-resistant species.

Aggregation of mPrP-(121–231) was markedly enhanced at 65 °C (corresponding to the Tm for thermal denaturation of this construct) with prompt appearance of aggregates within the first minutes of incubation at both pH 5.5 and 7.0. The subcellular localization of the conformational transition of PrPSc into PrPSc is still a matter of controversy. There is evidence indicating that it takes place both at the cell surface (at an approximate pH of 7.3 corresponding to the interstitial milieu of the brain; Refs. 34 and 35) and after internalization of PrP into endosomes (4, 24), where pH values range between 4.7 and 5.8 (36). In vitro, the conversion of human PrPSc to a PrPSc-like form is enhanced at acidic pH (37). Biophysical studies have shown that the free energy of unfolding of human PrP-(90–231) is lower at acidic...
pH than at neutral pH (26) and that in acidic guanidine hydrochloride solution human PrP-(90–231) adopts a partially folded conformation that contains a large amount of \( \beta \)-sheet secondary structure. A \( \beta \)-sheet-rich folding intermediate has also been observed for mouse mPrP-(121–231) in urea solutions at low pH but not at neutral pH (28). In addition, it has been shown that low pH induces an increase in the exposure of hydrophobic patches on the surface of mPrP-(121–231) (38). However, low pH alone is not sufficient to trigger the conformational transition of PrP. For example, incubation of hamster PrP-(90–231) at pH 4.0 at 4 °C for 50 h leads to the formation of amorphous aggregates and not amyloid structures (39). Additional destabilizing factors, such as subdenaturing concentrations of guanidine hydrochloride or urea in the presence of sodium chloride, have been shown to be required to promote the conformational transition of the prion protein. Our results show that incubation of mPrP-(121–231) at pH 4.0 and 65 °C for 1 week did not lead to prominent formation of amyloid fibrils. Rather, under those conditions globular aggregates and

![Image of electron microscopy analysis of aggregated mPrP-(121–231)](image)

**FIGURE 3.** Transmission electron microscopy analysis of aggregated mPrP-(121–231). The different panels show the formation of globular aggregates, protofibrils, and mature amyloid fibrils of mPrP-(121–231) upon incubation at 65 °C at different pH values. Left and right panels represent images obtained from samples incubated for 1 h or 7 days, respectively, at the following pH values: A and B, pH 5.5 (unbuffered solutions). A number of annular PrP aggregates can be seen upon close inspection of the image in A, and a representative example is shown at higher magnification as an inset; C and D, pH 7.0 (unbuffered solutions); E and F, pH 4.0 (buffered medium); G and H, pH 5.5 (buffered medium). The inset in H illustrates a high magnification field showing a bundle of amyloid fibrils meshed together; I and J, pH 7.0 (buffered medium). Scale bars correspond to 0.2 \( \mu \)m.

![Image of proteinase K digestion](image)

**FIGURE 4.** Limited proteinase K digestion of the amyloid form of mPrP-(121–231). Native monomeric mPrP-(121–231) (upper panel) or amyloid fibrils formed upon incubation of mPrP-(121–231) for 1 week at 65 °C and pH 7 (lower panel) were treated with proteinase K for 1 h at 37 °C at the PK:mPrP ratios indicated in the figure. Samples were analyzed by SDS-PAGE followed by silver staining. 3 \( \mu \)g of mPrP-(121–231) were applied in each lane. MW, molecular mass.
Oligomerization and Fibrillization of mPrP-(121–231)

protofibrillar structures, as well as some amorphous aggregates, were formed (Fig. 3F). Thus, these results suggest that pH < 5 and partially denaturing temperature favors the formation of PrP oligomers and protofibrils. Interestingly, samples incubated for only 1 h at pH 5.5 and 65 °C in unbuffered medium exhibited large numbers of oligomers of roughly spherical morphology forming clusters that resembled beads on a string (Fig. 3A). In addition, annular aggregates resembling those formed upon incubation of hamster PrP-(90–232) at pH 4.2 and 1 M guanidine hydrochloride for 25 days (40) were also observed (Fig. 3A, inset). Such annular structures have been proposed to consist of a ring of eight monomers of PrP (40).

Considering the increasing evidence that soluble non-fibrillar oligomers may play important roles in neurotoxicity in several other neurodegenerative diseases (41), an efficient experimental procedure to produce oligomers of the prion protein may help to identify the role played by such species in transmissible spongiform encephalopathies. In this regard, several groups have studied the conformational transition of different recombinant prion protein constructs into β-rich aggregates (40, 42–48). Some studies pointed to the formation of β-rich oligomers during the conformational transition of PrP (40, 48). It has been suggested that β-oligomers are not on the pathway to amyloid formation (42, 46) and that the preference for forming either a β-oligomer or an amyloid aggregate can be dictated by the experimental conditions with pH < 5 and partially denaturing concentrations of urea (4–5 M) favoring the β-oligomer and pH > 5 and low concentrations of urea (1–2 M) favoring amyloid (42). Our current findings indicate that the combination of elevated temperature and pH 5.5 in unbuffered medium is most effective in promoting the formation of mPrP-(121–231) oligomers. Moreover, under these experimental conditions, it appears that the oligomers are progressively consumed while protofibrils emerge, with longer incubation times leading to the disappearance of oligomers and the appearance of mature amyloid fibrils (Fig. 3, A and B).

The accumulation of SDS-resistant oligomers of mPrP-(121–231) is reminiscent of the formation of SDS-resistant oligomers of the β-amyloid peptide (49) and indicates the high stability of those species. It has been suggested that oligomerization of PrP can occur through the formation of intermolecular disulfide bonds (50, 51). However, oligomers of mPrP-(121–231) were not disassembled even by boiling in the presence of 140 mM dithiothreitol and SDS (Fig. 2). This suggests that the oligomerization of mPrP-(121–231) does not involve the formation of intermolecular disulfide bonds.

Treatment of PrPSc with PK generates a PK-resistant core encompassing residues 90–231, referred to as PrP27-30. The construct we have used does not contain the N-terminal domain of the prion protein that is cleaved by PK, and hence no change in molecular weight of the protein would be expected upon PK digestion. Nevertheless, incubation of mPrP-(121–231) at pH 7.0 at 65 °C for 1 week led to the formation of an abnormal aggregate characterized by enhanced resistance to PK digestion compared with native PrP (Fig. 4). These results suggest that, under these experimental conditions, mPrP-(121–231) aggregates into an amyloid that has physical properties of PrPSc.

In a recent study, Norstrom and Mastrianni (52) investigated the aggregation of a PrP construct in which a palindromic sequence corresponding to amino acid residues 112–119 in the hydrophobic core of the prion protein was deleted. The fact that this deletion mutant formed aggregates when expressed in yeast is in line with our current results and supports the notion that the C-terminal region of PrP may be sufficient for aggregation.

Synthetic prions generated in vitro have been shown to be similar to a newly identified subpopulation of PrPSc from sporadic Creutzfeldt-Jakob disease (53). Of significant interest, the amyloid form of this subpopulation contains a proteinase K-resistant core composed only of residues 152/153–230 and 162–230. Furthermore, an important recent study showed that conversion of PrPC to PrPSc and amplification of the latter can be achieved in vitro (54). Inoculation of wild-type hamsters with such in vitro-produced PrPSc led to a scrapie disease quite similar to the illness produced by inoculation of brain-derived infectious material (54).

In conclusion, the present work shows that an amyloid form with physical properties of PrPSc can be obtained in vitro using the C-terminal domain of PrP, which lacks the entire N-terminal flexible domain of PrP and most of the so-called amyloidogenic (hydrophobic) core of the protein. Understanding the conversion of PrP to an amyloid-specific, protease-resistant conformation may provide insight into the mechanisms of neurodegeneration and may lead to the development of novel strategies to combat prion diseases.

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