Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 368 (2008) 238–242
BBRC
www.elsevier.com/locate/ybbrc

Amyloidogenic properties of the prion protein fragment PrP(185–208): Comparison with Alzheimer's peptide Aβ(1–28), influence of heparin and cell toxicity

Marta Cortijo-Arellano [a], Jovita Ponce [b], Núria Durany [b], Josep Cladera [a,*]

[a] *Biophysics Unit and Centre of Studies in Biophysics, Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, 08193 Bellaterra, Spain*
[b] *Health Science Faculty, International University of Catalonia, Barcelona, Spain*

Received 7 January 2008
Available online 18 January 2008

Abstract

Amyloid fibrils are a hallmark of Alzheimer's and prion diseases. In both pathologies fibrils are found associated to glycosaminoglycans, modulators of the aggregation process. Amyloid peptides and proteins with very poor sequence homologies originate very similar aggregates. This implies the possible existence of a common formation mechanism. A homologous structural motif has recently been described for the Alzheimer's peptide Aβ(1–28) and the prion protein fragment PrP(185–208). We have studied the influence histidine residues and heparin on the aggregation process of both peptides and determined the possible amyloid characteristics of PrP(185–208), still unknown. The results show that PrP(185–208) forms amyloid aggregates in the presence of heparin. Histidines influence the aggregation kinetics, as in Aβ(1–28), although to a lesser extent. Other spectroscopic properties of the PrP(185–208) fragment are shown to be equivalent to those of other amyloid peptides and PrP(185–208) is shown to be cytotoxic using a neuroblastoma cell line.

Keywords: Prion; Alzheimer; Peptide; Amyloid; Fluorescence; Infrared

Amyloid diseases are related to anomalies in the folding processes of certain proteins involved in the formation of the so called amyloid fibrils, rich in β-sheet secondary structure [1]. The aggregation capacity of amyloid peptides and proteins depends on a conformational change which implies the conversion of unordered and α-helical structures into β-structures. This change is accepted to trigger the aggregation process, which shows typically sigmoidal kinetics, usually interpreted as describing a nucleation-dependent polymerization. Fibrillar aggregates are a hallmark of central nervous systems affected by different neurodegenerative diseases, such as Alzheimer's and prion diseases. Both are brain pathologies characterized by memory loss and impairment of cognitive functions (speech and motor skills). In both cases, fibrils are found in the affected tissues associated with some elements of the extracellular matrix, such as glycosaminoglycans (GAGs) [2]. It seems that GAGs would be able to modulate amyloid aggregation and a cluster of basic residues (VHHQK) has been proposed as a GAG binding motif [3,4]. Alzheimer's Aβ peptide aggregation has been shown to be enhanced by GAGs such as heparin, heparan sulfate, and others [2,3,5,6], whereas in the case of prion aggregation, inhibiting, and stimulating conditions have been described in the presence of GAGs [7,8]. As for the effects of these macromolecules on the development of the pathologies some controversy exists at present since both possible protective and enhancing effects have been described [9,10].

The fact that proteins and peptides with no sequence homology give place to proteinaceous aggregates with very similar physicochemical properties implies the existence of a possible common aggregation mechanism. Recently, a specific region has been identified as a possible

* Corresponding author. Fax: +34 935811907.
E-mail address: josep.cladera@uab.es (J. Cladera).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.01.049

a sphingolipid-binding motif, structurally homologous, in the fragment 1–28 of the Alzheimer's Aβ peptide and the fragment 185–208 of the human prion protein [11]. Since both sequences contain His residues and possible heparin binding motifs, we have used in the present work a combination of spectroscopic techniques (Fourier-Transform Infrared Spectroscopy and Fluorescence Spectroscopy) complemented with electron microscopy in order to compare the influence of heparin on the aggregation process of both peptides and further characterize PrP(185–208) as an amyloidogenic peptide.

The results show that PrP(185–208) is able to form amyloid aggregates following a nucleation-dependent polymerization only in the presence of heparin. Protonation of the His residues or their substitution by Ala is shown to slow down the aggregation process, as it happens for the Alzheimer's Aβ(1–28) peptide, although the effect in the case of the prion peptide is less pronounced. PrP(185–208) is shown to be cytotoxic using a neuronal cell line.

Materials and methods

Synthetic peptides Aβ(1–28), Aβ(1–28)H13A, Aβ(1–28)H14A, PrP(185–205), and PrP(185–205)H187A were purchased from JPT Peptides Technologies (Germany). Polypeptidic sequences are detailed in Table 1.

FT-IR measurements. Lyophilized peptides were dissolved in 10mM deuterated Hepes buffer and pD was adjusted with NaOD and DCl solutions (Sigma–Aldrich). The peptide concentration in the samples was always 1.2 mM, and heparin (Sigma–Aldrich) was added at 1 mg/ml. FT-IR spectra were recorded with a Mattson Polaris Fourier transform spectrometer at 37 °C at a resolution of 2 cm^{-1}. Samples were inserted between CaF_2 windows using a 50 μm Teflon spacer. The sample chamber was purged continuously with N_2 in order to remove water vapour. A corresponding deuterated buffer spectrum was subtracted from the spectrum of each sample.

Fluorescence measurements. The aggregation kinetics of the amyloid peptides were monitored using the dye Thioflavin T (ThT), which fluorescence is dependent on the formation of amyloid aggregates. Fluorescence measurements were carried out in a SLM-Aminco 8000 spectrofluorimeter. Excitation and emission wavelengths were set at 450 and 490 nm, respectively. Temperature was controlled with a thermostatic bath at 378 °C. The peptide concentration in the samples was always 50 μM, and heparin was added at 0.041 mg/ml.

MTT assay using SH-SY5Y neuroblastoma cells. Cells were plated at 37 °C on 96-well plates (Iwaki) at a density of 12,000 cells per well to confluency, with 5% CO_2 in a humidified atmosphere with Dulbecco's modified Eagle's medium (DMEM/Ham's F12, 1:1) with phenol red (Sigma). L-Glutamine (4 mM; Gibco), penicillin (100 U/ml; Gibco), streptomycin (100 μg/ml; Gibco), MEM non-essential amino acids (100×; Gibco) and 0.5% inactivated fetal bovine serum (FBS; Gibco) were added to the medium. The cells were incubated with peptide samples during 48 h. A 1/10 dilution of aqueous MTT solution (5 mg/ml) was then added to each well (100 μl), and the mixture was incubated at 37 °C for 90 min. After that time, medium culture was removed from the well-plate and the MTT reduction reaction was stopped adding 50 μl of DMSO in each well. The amount of formazan product was determined by measuring the absorbance in a 96-well ELISA plate reader at 595 nm. All MTT assays were duplicated.

Electron microscopy. Aggregated and non-aggregated samples from the fluorescence assay were taken at three different times and then were used in order to obtain electron micrographs. Ten microliters of these suspensions were placed on grids covered by a carbon film. Excess fluid was withdrawn after 1 min, and the grids were negatively stained with 2% uranyl acetate. The stained grids were then examined and photographed in a Hitachi H-7000 electron microscope at 70 kV.

Results

Effect of histidine substitutions on the aggregation of Aβ(1-28)

Fig. 1A and B illustrates how the substitution of either His13 or His14 in the Aβ(1–28) sequence, affects peptide aggregation at pH 5.5. Formation of fibrils was monitored using the fluorescent dye Thioflavin T, in the absence (panel A) and presence of heparin (panel B). Measurements of wild-type Aβ(1–28) in 10 mM Tris at pH 7.5 did not give any fluorescence increase within the monitored time interval (approximately 6 h, data not shown). At pH 5.5 and in agreement with previous reports [12], wild-type Aβ(1–28) readily aggregates, following, as clearly seen in the figure, the typical sigmoid-shaped amyloid formation kinetics, which is accelerated in the presence of heparin. In agreement as well with previous works [3] both in the absence and the presence of heparin, the H13A mutant fails to show any aggregation during the monitored time interval. H14A however does still aggregate, although more slowly, in the presence of heparin.

Fig. 2A–F shows the Fourier-transform infrared spectra of Aβ(1–28) and the His mutant sequences in the presence and absence of heparin at pD 5.5. For the infrared experiments the peptide concentration was 24 times higher than for the ThT experiments and at that peptide concentration (1.2 mM) the peptide is shown to aggregate very similarly under any of the tested experimental conditions: increase of the band at 1616 cm^{-1}, corresponding to the formation of aggregated β-structures [13] whereas the broad band centered at 1648 cm^{-1} (unordered and helical structures) decreases. The infrared data show therefore, that substituting either His 13 or His 14 for Ala does not preclude the formation of amyloid aggregates (they form when the concentration is increased 24 times), it just slows down the kinetics, as seen at low peptide concentration (ThT experiments). The electron micrographs shown in Fig. 3A–C,

Table 1
Alzheimer's Aβ(1–28) and prion PrP(185–208) sequences used in the present study

Aβ (1–28)	H_3^+N-DAEFRHDSGYEVHHQKLVFFAEDVGSNK-COO^-
Aβ (1–28)H13A	H_3^+N-DAEFRHDSGYEVAHQKLVFFAEDVGSNK-COO^-
Aβ (1–28)H14A	H_3^+N-DAEFRHDSGYEVHAQKLVFFAEDVGSNK-COO^-
PrP(185–205)	H_3^+N-KQHTVTTTTKGENFTETDVKMMER-COO^-
PrP(185–208)H187A	H_3^+N-KQATVTTTTKGENFTETDVKMMER-COO^-