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Full Length Research Paper

Effect of cadmium ions on amyloid beta peptide 1-42 channel activity

Notarachille Gabriella, Gallucci Enrico, Micelli Silvia and Meleleo Daniela*

Department of Pharmaco-Biology, University of Bari "Aldo Moro", via E. Orabona 4, 70126 Bari; Italy.

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Amyloid β-peptide (Aβ) is a natural peptide of about 39-42 amino acids, which can aggregate and accumulate into senile plaques, one of the main pathological features in Alzheimer's disease (AD). There is extensive evidence that neurodegenerative pathologies, such as AD, are associated with protein misfolding and environmental factors, such as heavy metals, that are known to pollute the environment and can be taken up by the organism in food. They can accumulate within organs and tissues, with sometimes dramatic effects. There is increasing evidence that heavy metals can interact with amyloid β peptides, contributing to the neurodegenerative events of AD. We investigated the effects of Cd^{++}, an environmental contaminant on AβP1-42 aggregation, incorporation and channel formation into planar lipid membranes made up of phosphatidylcholine: cholesterol (70:30, w/w). Our results suggest that Cd^{++} interferes both with channels already incorporated into membranes and with peptides in solution. These findings provide important clues to the effect of this environmental contaminant on AβP1-42 that similarly to other metal ions, such as copper, zinc, aluminium and iron, can lead to abnormal interactions with proteins, contributing to cell damage.

Key words: Amyloid β-peptide (AβP) 1-42, cadmium, planar lipid membrane, ion channel.

INTRODUCTION

Many neurodegenerative disorders, such as Alzheimer's disease (AD), as well as certain systemic diseases are associated with protein misfolding and abnormal aggregation into organs and tissues, causing cellular dysfunctions, that culminate in cell death (Lal et al., 2007).

AβP1-42 is a small amphiphilic peptide with a hydrophilic N-terminal domain (residues 1-28) and a hydrophobic C-terminal (residues 29-42) (Selkoe, 2004), and is considered to be the more toxic form of AβP responsible for the pathogenesis of Alzheimer's disease (Selkoe, 2001a, b).

Normally AβP is released into the body fluids, where it can remain in a non-toxic and non-aggregated state. Therefore the peptide's conversion from a native soluble form to a non-native insoluble one, such as small and large aggregates, protofibrils and fibrils of AβP enriched in β-sheet structures, is implicated in the pathogenesis of AD. Although the etiologic role of AβP1–42 in Alzheimer's disease is accepted, the molecular mechanism of neurotoxicity remains a matter of debate: AβP oligomers can increase the area per molecule of the membrane-forming lipids, thus thinning the membrane, lowering the dielectric barrier and increasing conductance (Sokolov et al., 2006); or by interacting with the membrane, AβP can insert into natural or artificial membranes in a configuration capable of forming ion channels (Ambroggio et al., 2005; Arispe, 2004; Arispe et al., 2007; 1993a; 1993b; Capone et al., 2009; Díaz et al., 2006; Jang et al., 2008; Kagan et al., 2004; Kawahara et al., 1997; Kourie and Henry, 2001; Lal et al., 2007; Lin et al., 2001; Micelli et al., 2004; Quist et al., 2005; Vestergaard et al., 2008) which in turn alter the membrane's ionic permeability.

Recently, several studies indicate that metal ions, such as zinc, copper and iron, play an important role in the promotion of AD, but the specific mechanism of their toxicity remains to be elucidated (Adlard and Bush, 2006; Tõugu et al., 2008). Although alterations to the metal

*Corresponding author. E-mail: dmeleleo@farmbiol.uniba.it. Tel: +390805442775. Fax: +390805442775

metabolism do occur to some extent in normal ageing, they appear to be highly enhanced under various neuropathological conditions, causing increased oxidative stress and favouring abnormal metal-protein interactions. Cadmium is a long-lived toxic heavy metal used in industry for pigments, batteries, plastics and metal coatings. The most common sources of environmental cadmium exposure are contaminated food, water and cigarettes. Pan and colleagues (Pan et al., 2010) report cadmium levels in Europe and its implications for human health. The amounts of cadmium adsorbed from humans depend on the cadmium concentration in air, contaminated food and the individual habits.

Several studies highlight the severe neurotoxic effects of this metal, in the concentration range of 50-500 µM, on the function of the central nervous system, affecting behaviour, development, and neurotransmission and on the peripheral nervous system, damaging nerve cells and nerve fibres (Moschou et al., 2008).

In particular, it has been observed that cadmium levels in the bodies of Alzheimer's disease patients, compared with 'normal' subjects, were significantly elevated, especially in liver (Lui et al., 1990), plasma (Basun et al., 1991) and brain tissues (Panayi et al., 2002).

The aim of this work was to study the ability of AβP1-42 to incorporate and form ion channels in a planar lipid membrane (PLM) made up of phosphatidylcholine:cholesterol (POPC:Chol = 70:30, w/w), a composition similar to that of a neuronal membrane, in the absence and in the presence of Cd++ by means of the voltage-clamp technique.

METHODS

Single-channel measurements

Channel activity was recorded in lipid bilayer membranes (PLMs) made up of POPC:Chol (70:30, w/w) (SIGMA) in 1% n-decane (FLUKA), prepared as previously described (Micelli et al., 2004). Bilayers were formed across the 300 µm diameter of a circular hole in a teflon partition separating two teflon chambers containing symmetrical KCl 0.1M solutions by the "painting" technique, as described elsewhere (Benz et al., 1978), pH=7, temperature 23±1°C. The salts used in the experiments were of analytical grade.

The solution of AβP1-42 (SIGMA) was prepared by dissolving AβP powder in bidistilled sterile water and further diluted in bidistilled sterile water; then the solution obtained was stored at -20°C until use.

The final concentrations of AβP1-42 and of $CdCl_2$ (SIGMA) chosen in all series of experiments were $5 \cdot 10^{-8}$M and $2.5 \cdot 10^{-4}$M, respectively.

In single-channel experiments, the membrane current was monitored with an oscilloscope and recorded on a chart recorder for further data analysis by hand. The *cis* and *trans* chambers were connected to the amplifier head stage by Ag/AgCl electrodes in series with a voltage source and a highly sensitive current amplifier. The single-channel instrumentation had a time resolution of 1-10 msec, depending on the magnitude of the single-channel conductance. The polarity of the voltage was defined according to the side where AβP was added (the *cis* side). A *trans*-negative potential (indicated by a minus sign) means that a negative potential was applied to the *trans* side, the compartment opposite the one where AβP was added.

First of all, in order to monitor the AβP1-42 channel activity, the experiments were carried out in the presence of peptide alone (control condition); then three different experimental procedures were performed in the presence of $CdCl_2$:

1. In the first series of experiments, AβP1-42 was added to the *cis* side of the membrane. After channel formation, in the open-channel state, $CdCl_2$ was added to the same side as AβP;
2. In the second series of experiments, after AβP1-42 channel formation, in the open-channel state, $CdCl_2$ was added to the *trans* side of the membrane.
3. In the third series of experiments, AβP1-42 was pre-incubated with $CdCl_2$ and then this mixture was added to the *cis* side of the membrane. In these experiments, the peptide-cadmium mixture was prepared by diluting 6.06µl of AβP1-42 $4.6 \cdot 10^{-5}$M in 13.94 µl of $CdCl_2$ 0.1 M, under stirring for 2 min. The incubation times were 1h, 1h 30', 6 and 24 h, respectively. Once the bilayer was formed, the peptide-cadmium mixture, carefully stirred for 2 min, was added to the *cis* chamber.

Data analysis

The average conductance ($\bar{\Lambda}$)was determined by recording single events obtained from at least three experiments performed on different days for each experimental procedure and averaging over the distribution of the conductance values (Ludwig et al., 1986).

The channel-occurrence frequency, i.e. the mean number of openings in a period of 60 s, obtained from the total number of records, was calculated for each applied voltage in all tested conditions.

To define the channel lifetime, the channel durations were measured considering the time between the opening and closing of each channel. The average lifetime of the conductance unit was estimated by the formula:

$$N = A_1 e^{(-t/\tau 1)} + A_2 e^{(-t/\tau 2)}$$

where N is the number of channels that remain open for a time equal to or greater than a certain time t, A_1 and A_2 are the zero time amplitudes, and τ_1 and τ_2 are related to the fast and slow components of the time constant, respectively. The single-exponential distribution is included in the formula (A_2=0). To choose between the two models, we performed an appropriate statistical test (F-test, GraphPad Prism™ version 3.0; GraphPad Software, Inc., http://www.graphpad.com).

Circular dichroism measurement

CD spectra were recorded on a Jasco J-715 spectropolarimeter at room temperature. Cells with a path length of 0.1 cm were used for spectra recorded between 195 and 260 nm, with sampling points every 0.2 nm.

The AβP1-42 stock solution concentration was $5.77 \cdot 10^{-5}$ M. The aqueous buffer used to dissolve the peptide was 0.1 M KCl pH 7. Peptide samples were prepared from stock solution at a concentration of $5.77 \cdot 10^{-6}$ M in 0.1 M KCl pH 7. Samples containing cadmium were prepared by adding $CdCl_2$ at a final concentration of $2.5 \cdot 10^{-4}$ M to the AβP1-42 samples. CD spectra were recorded 5 min, 24 and 48 h after preparing the samples. Each CD spectrum consisted of four consecutive scans at a scanning speed of 20 nm/min. The samples were stored at room temperature for

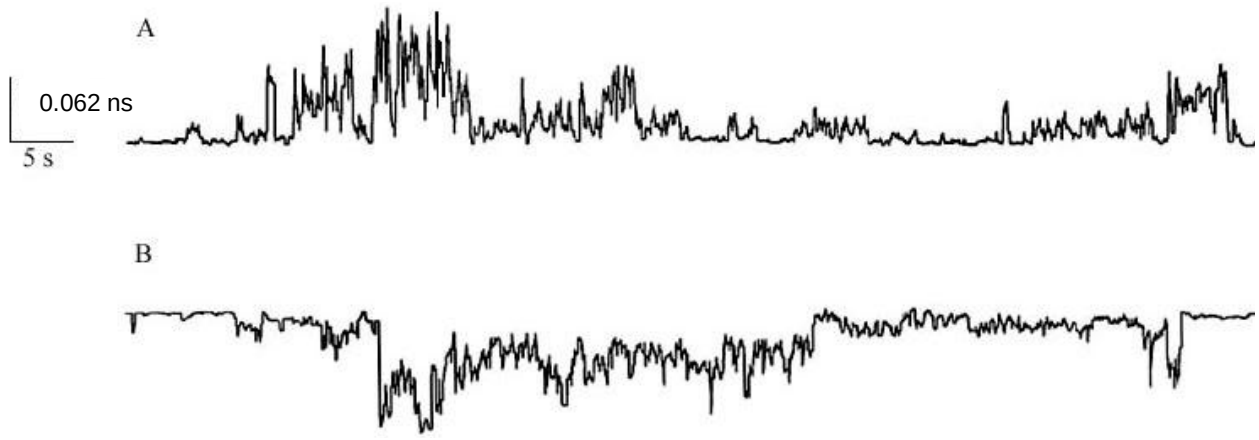

Figure 1. Examples of chart recordings of the A**β**P1-42 channel activity incorporated into PLMs made up of POPC: Chol (70:30, w/w) at an applied voltage of 80 mV (A) and -80 mV (B), respectively. Each trace represents a fragment of the recording of the activity obtained in individual experiments. Note the increased activity of the channel and the frequent multiple levels of conductance. Experimental conditions: KCl 0.1M; A**β**P1-42 $5 \cdot 10^{-8}$M was present on the *cis* side of the membrane

(subsequent analysis).

RESULTS

AβP1-42 channel activity in POPC: Chol PLMs

First of all, in each experiment we tested the stability of the membrane by applying a voltage of ± 120 mV for 10-15 min under stirring, and monitoring constant values for PLM conductance and capacitance. Neither conductance nor capacitance ever exceeded 12.5 pS and 0.23 $\mu F/cm^2$, respectively, and showed no channel-like activity.

In a preliminary set of experiments, after membrane formation and stabilization, AβP1-42 was added to the *cis* side of the medium facing the membrane and AβP1-42 conductance was monitored.

In all experiments, the addition of $5 \cdot 10^{-8}$ M of AβP1-42 to the *cis* side of the medium facing the membrane did not determine any conductance variation over a period of some hours, even upon application of voltages as high as ±120 mV. However, after PLM breakage and withdrawal, AβP1-42 channel activity appears, at an applied voltage of ±100 mV, as non-random discrete current jumps, that fluctuate between conductive and non-conductive states, compatible with channel-type opening and closure, with different conductance levels, occurrence frequencies and lifetimes, indicating AβP1-42 incorporation into PLM.

Over time (not less than 20 h), it was observed that AβP1-42 channel frequency, lifetime and current jump amplitudes increase, allowing the applied voltage to be lowered to ±40 mV. For applied voltages higher than ±100 mV, the channel activity was extremely intense, becoming paroxystic and causing membrane breakage. It is noteworthy that the number of open events is always greater than the number of terminating events (channels) observed.

Figure 1 shows an example of chart recordings of the AβP1-42 channel activity incorporated into the PLM at an applied voltage of ±80 mV.

A histogram of the average conductance amplitude distribution at each applied voltage is reported in Figure 2A. The data of the voltage-conductance $\bar{\lambda}$ relationship of AβP1-42 show that $\bar{\lambda}$ is inversely correlated with applied voltage to the membrane. Figure 2B reports the histogram of the AβP1-42 occurrence frequency ± standard deviation at the different applied voltages. The results obtained indicate that the occurrence values in the applied voltages range between -60 and +60 mV are significantly higher than those at ± 80 and ± 100 mV. This behaviour depends on the paroxystic channel activity at the applied voltages of ± 80 and ± 100 mV during which it is impossible to perform a rigorous analysis of the real number of channels.

The single-channel current recordings with not less than 100 channels were analyzed to obtain cumulative open-state lifetime distributions. Open times of channels, obtained at each voltage and in all experimental conditions, were fitted with a single- or two-exponential function obtaining one or two constants K_1 and K_2, that are used to calculate one lifetime (τ) or two lifetimes (τ_1, τ_2), respectively. Table 1 shows that AβP1-42 channel lifetime present two channel populations at each applied voltage, which, as is known, indicates stability of the AβP 1-42 channel.

Effects of cadmium on bare membrane and AβP1-42 channel conductance

To exclude any non-specific and destabilizing effects of

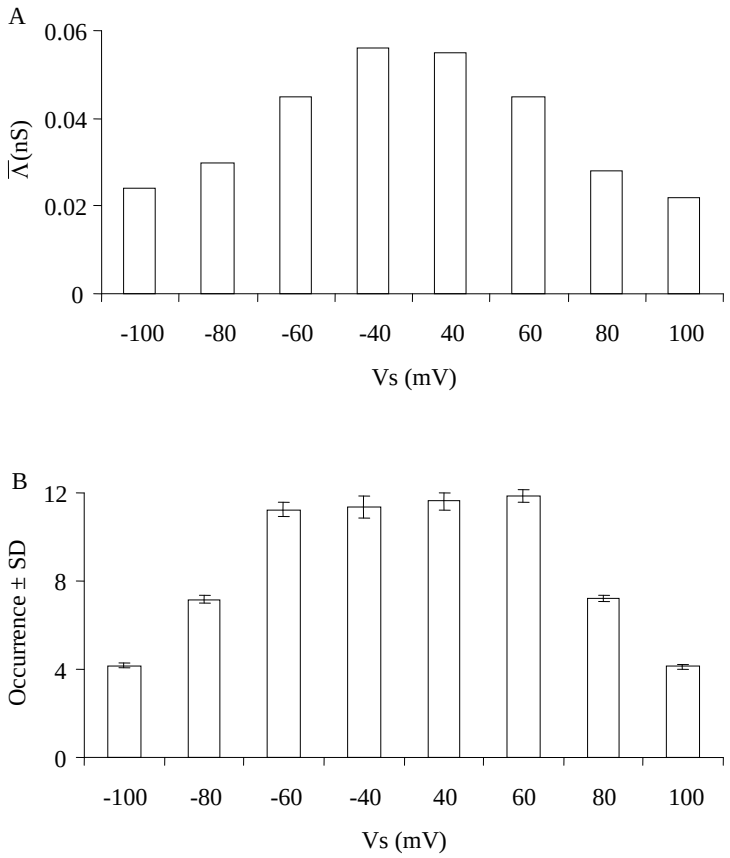

Figure 2. A**β**P1-42 channel conductance and frequency in POPC: Chol (70:30, w/w) PLM in control conditions: (A) the average conductance $\overline{\Lambda}$ (nS) and (B) occurrence frequency ± SD of AßP1-42 channel at each applied voltage.

Table 1. AβP1-42 channel lifetimes in POPC: Chol PLM.

Vs (mV)	AβP1-42 control		
	τ_1 (s)	τ_2 (s)	N_{ch}
100	1.377	7.374	1363
80	1.152	7.639	1868
60	1.407	10.865	1109
40	0.854	4.860	567
-40	1.342	15.320	345
-60	1.193	8.058	730
-80	1.332	10.621	1145
-100	1.202	6.313	1044

The fitted lifetimes of the AβP 1-42 single-channel in POPC: Chol (70:30, w/w) PLMs at different applied potentials in control conditions

cadmium *per se* on the PLMs used, we performed experiments, at an applied voltage of ±100 mV, by adding cadmium to the medium facing the membrane, and monitoring the PLM conductance and capacitance. Cadmium did not cause any variations in membrane conductance and capacitance over a long period of time (about 7 h). Different sets of experiments in the presence of cadmium were performed.

In the first set of experiments after AβP1-42 channel formation, in the open-channel state, Cd^{++} was added to

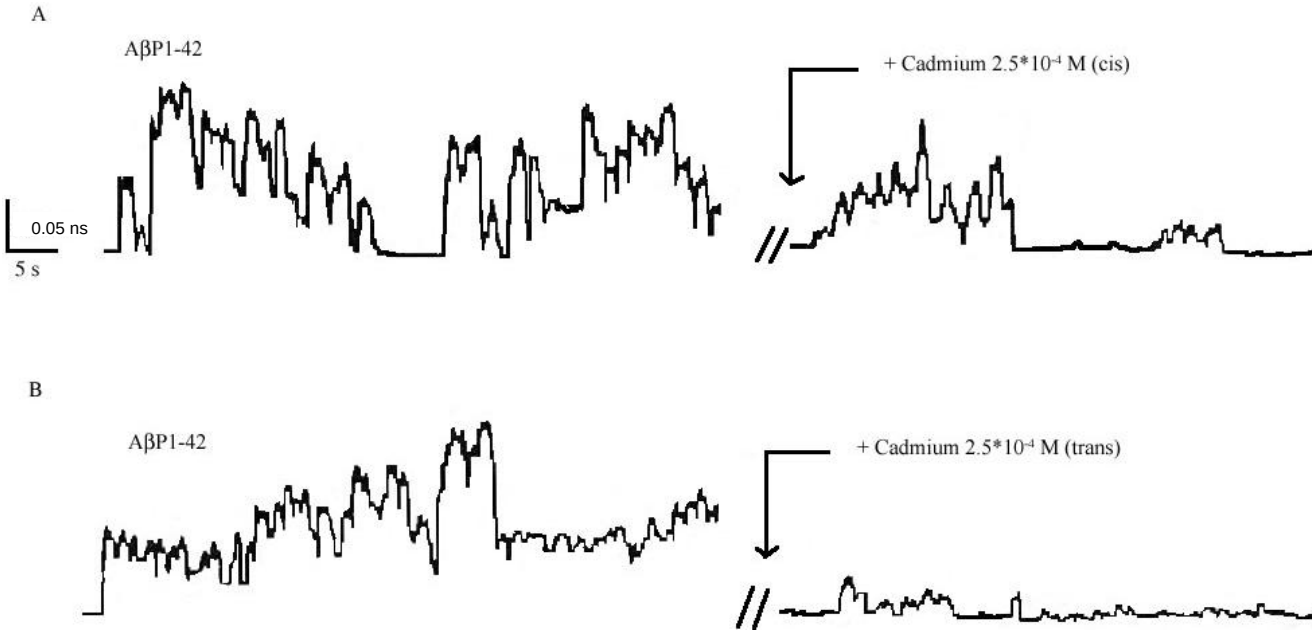

Figure 3. Examples of chart recordings of A**β**P1-42 channel activity, incorporated into PLMs made up of POPC: Chol (70:30, w/w), in the absence and presence of cadmium, at an applied voltage of 100 mV. A**β**P1-42 before and after $CdCl_2$ addition to the *cis* side (A); A**β**P1-42 before and after $CdCl_2$ addition to the *trans* side (B). Each trace represents a fragment of recording of activity obtained in individual experiments. Experimental conditions: KCl 0.1M; pH=7; A**β**P1-42=$5 \cdot 10^{-8}$M; $CdCl_2 = 2.5 \cdot 10^{-4}$M

the *cis* chamber at an applied voltage of 80 mV; in almost all the experiments, Cd^{++} addition soon leads to breakage of the membrane; a second membrane was immediately formed by painting the lipid solution present around the hole, and channel conductance was monitored.

In the presence of Cd^{++} on the *cis* side of the medium facing the membrane, AβP1-42 channel activity is characterized by lower conductance levels, occurrence frequencies and lifetimes than for the AβP1-42 channel alone. It can be seen that the channel activity progressively decreases until it completely disappears after a mean time of about 2 h and 30 min (Figure 3A).

In a second set of experiments, after AβP1-42 channel formation, in the open-channel state, Cd^{++} was added to the *trans* chamber at an applied voltage of 80 mV. Also in this series, cation addition soon causes destabilization and frequent membrane breaks; then, using the same protocol described before to form a new membrane, the channel conductance was monitored. The presence of Cd^{++} on the *trans* side leads to a more drastic reduction in AβP1-42 conductance levels and occurrence frequency than cation addition to the *cis* side of the medium: in fact, after a lag time of about 90 minutes, the channel activity completely disappeared (Figure 3B).

In a third set of experiments, AβP1-42 was preincubated with Cd^{++} for different times: namely 1 h, or 1 h 30 min, or 6 h, or 24 h; subsequently, each peptide-cation mixture was added to the *cis* side of the membrane at an applied voltage of 80 mV. No channel activity of the peptide was observed at a voltage of 80 mV applied for two hours. AβP1-42 preincubated with Cd^{++} needed a higher applied voltage (±100 mV) to form a channel, although this figure is lower than all of the experimental sets described before.

It is worth noting that the channel activity of the AβP1-42 preincubated with Cd^{++} at 6 h was so low that it failed to provide any conspicuous data, thus no rigorous analysis of the conductance, occurrence frequency and lifetime could be performed. The channel activity disappeared completely when the preincubation time exceeded 6 h (Figure 4).

In the presence of cadmium on the *cis*- or *trans*-side, the inhibiting effect of the cation does not allow analysis of the voltage-conductance relationship to be performed; only at an applied voltage of ±80 and ±100 mV is it possible to observe channel events in which $\bar{\Lambda}$ is not modified compared to that of AβP1-42 alone. In preincubation conditions, the channel activity was also reduced when the applied voltage decreased to under ±100 mV and disappeared at each applied voltage in preincubation lasting 24 h. The average conductance ($\bar{\Lambda}$) is reported in Table 2 for all tested conditions.

AβP1-42 channel occurrence and lifetime in the absence and presence of $CdCl_2$

The AβP1-42 occurrence frequency is characterized by high channel turnover at applied voltages in the range of

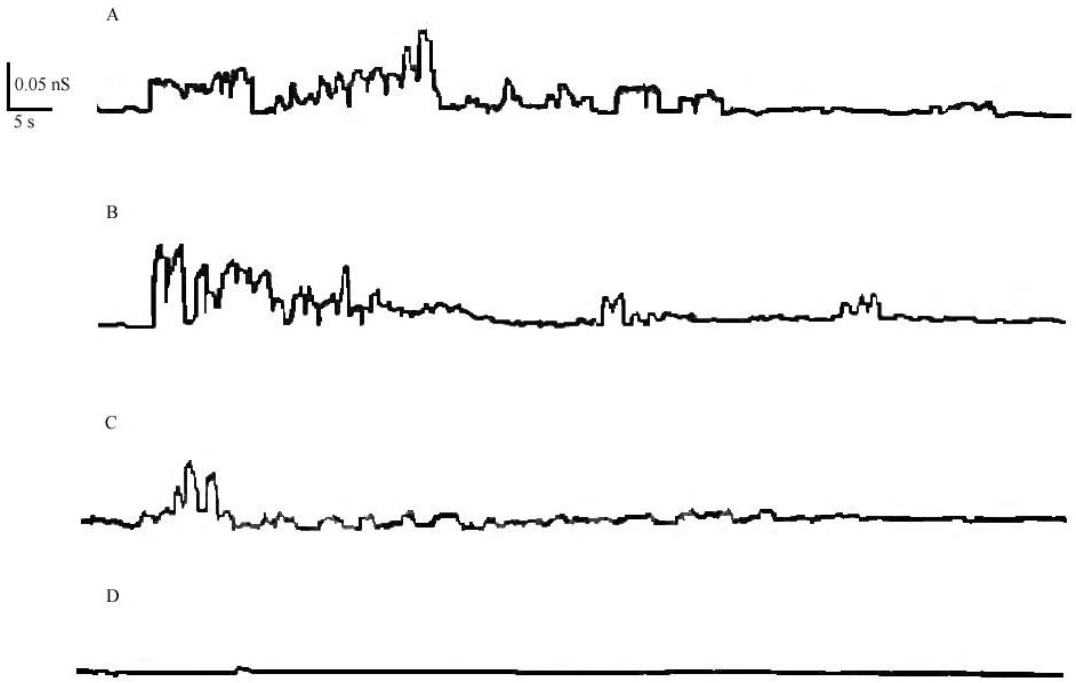

Figure 4. Representative channel activity of A**β**P1-42 preincubated with $CdCl_2$ for 1 h (A), for 1h and 30'(B), for 6 h (C), for 24 h (D), at an applied voltage of 100 mV, incorporated into PLMs made up of POPC:Chol (70:30, w/w). Each trace represents a fragment of recording of activity obtained in individual experiments. Experimental conditions: KCl 0.1M; pH=7; A**β**P1-42=$5 \cdot 10^{-8}$M; $CdCl_2 = 2.5 \cdot 10^{-4}$M.

Table 2. AβP1-42 channel conductance in POPC: Chol PLM.

mode	**AβP1-42 control**	**AβP142+Cd^{++} added to the *cis* side**	**AβP142+Cd^{++} added to the *trans* side**	**AβP142+Cd^{++} preincubated 1h**	**AβP142+Cd^{++} preincubated 1h 30'**
Vs (mV)	**$\bar{\Lambda}$ (nS)**	**$\bar{\Lambda}$ (nS)**	**$\bar{\Lambda}$ (nS)**	**$\bar{\Lambda}$ (nS)**	**$\bar{\Lambda}$ (nS)**
100	0.022	0.020	0.016	0.021	0.018
80	0.028	0.028	0.026	---	---
-80	0.030	0.024	0.023	---	---
-100	0.024	0.020	0.022	0.021	0.020

The average conductance $\bar{\Lambda}$ (nS) of AβP1-42 channels in POPC: Chol (70:30, w/w) PLMs at different applied voltages in control conditions and in the presence of Cd^{++} added to the *cis* or *trans* side of the PLM and preincubated with the peptide. --- = no interaction

±40 ÷ ±100 mV (Figure 2B). By contrast, in the presence of cadmium, AβP1-42 channel activity was not registered at voltages under ±80 mV.

In Table 3, to make a comparative analysis, the occurrence frequency values are reported at the applied voltages of ±80 and ±100 mV. It can be seen that after channel insertion, when cadmium was added to the *cis* side, the occurrence frequency decreases and that this effect is markedly more evident when cadmium was added to the *trans* side of the membrane containing the channel.

When Cd^{++} is preincubated with peptide, the occurrence decreases as the incubation time increases (Figure 4); furthermore, a decrease in applied voltage to under ±100 mV causes the channel to disappear.

These data indicate that the addition of Cd^{++} leads to a decrease in channel turnover that culminates in a drastic blockage of channel activity.

Furthermore, it must be considered that the strong inhibitory effect of Cd^{++} on channel activity does not allow a lifetime analysis to be performed. Table 4 shows the minimum and maximum number of channels obtained (N) out of a total number of channels observed (Nt) in a series of experiments performed.

Table 3. A**β**P1-42 occurrence frequency in POPC: Chol PLM.

mode	AβP1-42 control	AβP142+Cd^{++} added to the *cis* side	AβP142+Cd^{++} added to the *trans* side	AβP142+Cd^{++} preincubated 1h	AβP142+Cd^{++} preincubated 1h 30'
Vs (mV)	**Occurrence** ±SD	**Occurrence ±SD**	**Occurrence** ±SD	**Occurrence** ±SD	**Occurrence** ±SD
+100	4.119±0.084	1.02±0.01	0.04±0.02	0.49±0.05	0.11±0.03
+80	7.20±0.127	1.44±0.11	0.57±0.06	---	---
-80	7.158±0.166	1.13±0.03	0.69±0.11	---	---
-100	4.164±0.100	0.23±0.05	0.14±0.04	1.19±0.04	0.21±0.05

Occurrence frequency ± standard deviation (occurrence ± SD) of A**β**P1-42 in the absence and in the presence of Cd^{++} in all tested conditions at applied voltages of ±80mV and ±100mV.
--- = no interaction

Table 4. A**β**P1-42 channels number in different experimental conditions.

Mode	Minimum and maximum number of channels	Nt
AβP1-42	345<N<1868	8171
AβP1-42+Cd^{++} to the *cis* side	5<N<84	164
AβP1-42+Cd^{++} to the *trans* side	4<N<58	89
AβP1-42+Cd^{++} preincubated 1h	10<N<31	41
AβP1-42+Cd^{++} preincubated 1h 30'	5<N<7	12

The minimum and maximum number of channels obtained (N) out of a total number of channels observed (Nt) at all applied voltages in different series of experiments performed.

Effects of cadmium on AβP1-42 secondary structure

Many conformational studies have documented that, depending on the solvent property, the peptide can also undergo a conformational transition *in vitro* (Tomaselli et al., 2006). AβP is present in an α-helix structure in a membrane or membrane-mimicking environment such as ionic detergents. In contrast, AβP exists mainly as a random coil and/or β-sheet conformations in aqueous solution (Xu et al., 2005).

To test whether cadmium modifies the secondary structure of AβP1-42, CD experiments using AβP1-42 samples in the absence and presence of cadmium were carried out. The samples containing AβP1-42 alone represent the control condition. Figure 5 shows the CD spectra of AβP1-42 without and with Cd^{++} measured after 5 min (T0), 24 h (T24) and 48 h (T48).

The features of spectra of AβP1-42 alone (Figure 5A) indicate that the AβP1-42 conformation, predominantly β-sheet and random-coil in an aqueous environment, modifies over time. At T48, the signal intensity decreases not only compared to T24 but also compared to T0, indicating a possible state of aggregation. The presence of Cd^{++} does not seem to modify the conformation of the peptide, but rather appears to stabilize the secondary structure. In fact, the CD spectra (Figure 5B) show that no change in signal intensity is observed at T0, T24 and T48.

Further studies on the structural analysis of AβP1-42 in the absence and presence of cadmium are needed to supplement and clarify some aspects of research.

DISCUSSION

It has been proposed that the cellular damage and degeneration induced by AβP in Alzheimer's disease may occur either by the interaction of the soluble oligomers of AβP with the neuronal membrane (Demuro et al., 2005) or by the formation of harmful AβP ion channels (Arispe et al., 1994a, b), that by altering the membrane permeability create an ion imbalance, which may induce the activation of intracellular events, responsible for cell degeneration.

Our results demonstrate that AβP1-42 incorporates and forms voltage-dependent ion channels into PLMs made up of POPC: Chol, confirming previous studies on the ability of AβP1-42 to form transmembrane ion channels (Bhatia et al., 2000; Hirakura et al., 1999; Micelli et al., 2004; Rhee et al., 1998). In the process of incorporation and channel formation, the lipid composition of the membrane and the AβP aggregation state assume a fundamental role. It has been demonstrated that the addition of 33% cholesterol to POPC membranes induces a conformational conversion of peptide in the α-helical structure that favours incorporation into the membrane (Ji et al., 2002; Micelli et al., 2004; Yip et al., 2002).

On the other hand, the AβP1-42 incorporation into

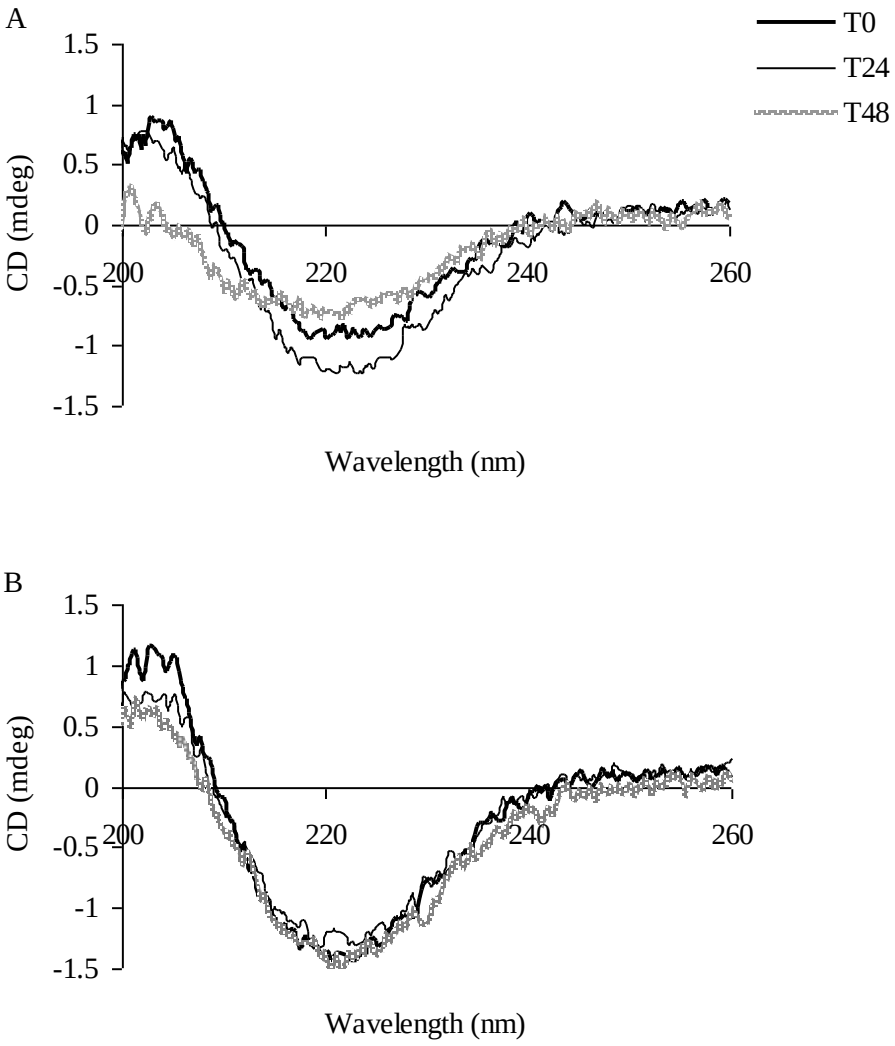

Figure 5. A**β**P1-42 secondary structure. Far-UV CD spectroscopy of A**β**P1-42 ($5.77 \cdot 10^{-6}$M) in the absence of cadmium (A). Far-UV CD spectroscopy of A**β**P1-42 ($5.77 \cdot 10^{-6}$M) in the presence of cadmium ($2.50 \cdot 10^{-4}$M) (B). The CD spectra were measured after 5 minutes (T0), 24 h (T24) and 48h (T48). Experimental conditions: KCl 0.1M; pH=7; A**β**P1-42=$5.77 \cdot 10^{-6}$M; $CdCl_2 = 2.5 \cdot 10^{-4}$M.

POPC: Chol PLM is not spontaneous and channel activity appears after the PLM breakage and withdrawal. The withdrawal by "brushing the torus" might indicate that the AβP1-42 in contact with lipids on the torus had been folded as a channel units. This mechanism has been proposed for others peptides and proteins (Gallucci et al., 1996; Micelli et al., 2000).

Also, it has been shown that experimental, biological and environmental factors, such as temperature, pH and metal ions, can influence the conformational structure of AβP and consequent interactions with the membrane. Furthermore, recent studies have reported that some metals are able to accelerate the dynamics of Aβ aggregation, increasing the neurotoxic effects on neuronal cells as a consequence of marked biophysical alteration of peptide properties (Drago et al., 2008). High concentrations of metal ions (copper, zinc, iron) have been found in amyloid deposits in the human brain (Bush, 2003; Lovell et al., 1998). Because an extensive literature has demonstrated the potential involvement of cadmium in cognitive impairment and other processes that may contribute to neurodegeneration (Adlard and Bush, 2006; Bojarski et al., 2008; López et al., 2006; Mattson, 2007), our research concentrates on studying the effects of this cation on the aggregation state, incorporation and channel activity of AβP1-42, which plays a key role in the pathogenesis of AD. The cadmium concentration used in this study is in the range of 50-500 µM tested on the nerve cells and nerve fibres (Moschou et al., 2008).

Cadmium is an occupationally and environmentally important toxic element that is present naturally in rocks, soil and sediment, but also occurs in air, water and

foodstuffs. Non-occupational exposure is mainly from diet and smoking with an estimated individual daily consumption of 30 µg in the USA and higher in China and Japan (Joseph, 2009; Satarug and Moore, 2004). Cadmium is efficiently retained in the organism and remains accumulated in the human body for life; its targets in terms of toxicity are lung, liver, kidney, bone, cardiovascular system and immune system, inducing loss of function and cell death (Templeton and Liu, 2010). Several studies have demonstrated that cadmium also tends to accumulate in the central nervous system, inducing a variety of neurotoxic effects, including behavioural, histopathological and neurochemical alterations (Fernández-Pérez et al., 2010; Matés et al., 2010; Moschou et al., 2008).

In particular, a cadmium concentration of 250 µM was used to mimic the effects of acute or chronic occupational and environmental metal exposure. Our results indicate that cadmium is able to influence AβP1-42 channel activity, by acting on the channel incorporated into the membrane as well as on the peptide in solution, both decreasing AβP1-42 channel activity and turnover until the channel is completely blocked, although in solution Cd^{++} seems to stabilize the AβP structure (CD data). The data shows that Cd^{++} acts more rapidly when added to the Trans side of the membrane than when added to the cis side. These results provide additional evidence for asymmetric AβP channels into lipid membranes, consistent with previous studies (Arispe et al., 1996; Vargas et al., 2000) and with the channel model proposed by Durell et al. (1994). Probably, after incorporation and channel formation, the binding-site(s) for Cd^{++} arrange in such a way as to form a cap that preferentially obstructs one site (trans) of the AβP1-42 channel. It can be suggested that cadmium, like zinc, copper and iron, is able to coordinate the Aβ peptide, through the histidine residues at positions 6, 13 and 14 and the tyrosine residue at position 10, located at the N-terminal part of the peptide. This binding could obstruct the AβP ion channel.

Moreover, data obtained when Cd^{++} was preincubated with AβP suggest that cadmium also acts on the peptide in solution, leading to a possible conformational variation incompatible with incorporation into the membrane. Alternatively, it could be suggested that cations could interact with specific groups of AβP1-42 that alter the folding of the peptide. In order to probe for possible conformational changes and investigate the secondary structure of AβP1-42, CD spectroscopy was used. Spectra obtained in the presence of Cd^{++} show that cadmium stabilizes the AβP1-42 structure in the disaggregated state, counteracting the peptide's native tendency toward aggregation (Figure 5).

It could be suggested that the presence of the cation alters the AβP1-42 folding responsible for the reduced or failed channel formation across the membrane. This altered folding could over time favour peptide assembly into small “amorphous” aggregates (Glabe, 2008). In fact, small aggregates have been isolated from the brain of AD patients (Gong et al., 2003) and seem to be essential for toxicity and cognitive dysfunction (Deshpande et al., 2006; Lesné et al., 2006).

Numerous research teams have demonstrated *in vitro* and *in vivo* that oligomeric AβP aggregates have higher neurotoxicity than fully mature amyloid fibrils (Dahlgren et al., 2002; Deshpande et al., 2006; Resende et al., 2008). AD symptoms such as memory and cognitive deficits, as well as synaptic loss, correlate better with the amounts of soluble Aβ oligomers compared to insoluble plaques in the brain of AD patients, as well as in mouse models (Haass and Selkoe, 2007). So, it cannot be excluded that the presence of the metal could promote a misfolding of the peptide that can be subtracted by channel formation across the membrane, but also a change in the charge of the peptide and modifications to the biophysical features of the membrane. These changes might modify electrostatic peptide-membrane interactions and thus also channel formation (Karabaliev, 2007).

The literature is full of data on increased levels of metals in critical brain areas of neurodegenerative disease sufferers, such as AD patients (Migliore and Coppedè, 2009a). Metal ions play an important role in AD, acting as mediators of events that culminate with cell degeneration. This is consistent with recent studies in vitro and in vivo demonstrating that metals, such as zinc, copper, iron and aluminium, are directly involved in the pathogenesis of AD, by affecting protein aggregation, oxidative damage and processes that are crucial in the normal functioning of the central nervous system (Adlard and Bush, 2006; Migliore and Coppedè, 2009b).

Therefore, compounds with chelation properties and also with the ability to block binding sites could be useful in the treatment of Alzheimer’s disease. Owing to the complexity of the interplay between the peptide and metals, and the high potential toxicity caused by the high environmental impact and long biological half-life of cadmium, further studies and structural analysis of AβP1-42 in the presence of this cation are continuing in the hope of clarifying some aspects of this interaction.

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