Aβ(31–35) and Aβ(25–35) fragments of amyloid beta-protein induce cellular death through apoptotic signals: Role of the redox state of methionine-35

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Abstract In order to clarify the basis of neuronal toxicity exerted by the shortest active peptides of amyloid beta-protein (Aβ), the toxic effects of Aβ(31–35) and Aβ(25–35) peptides on isolated rat brain mitochondria were investigated. The results show that exposure of isolated rat brain mitochondria to Aβ(31–35) and Aβ(25–35) peptides determines: (i) release of cytochrome *c*; (ii) mitochondrial swelling and (iii) a significant reduction in mitochondrial oxygen consumption. In contrast, the amplitude of these events resulted attenuated in isolated brain mitochondria exposed to the Aβ(31–35)Met35^{OX} in which methionine-35 was oxidized to methionine sulfoxide. The Aβ peptide derivative with norleucine substituting Met-35, i.e., Aβ(31–35)Nle-35, had not effect on any of the biochemical parameters tested. We have further characterized the action of Aβ(31–35) and Aβ(25–35) peptides on neuronal cells.

Taken together our result indicate that Aβ(31–35) and Aβ(25–35) peptides in non-aggregated form, i.e., predominantly monomeric, are strongly neurotoxic, having the ability to enter within the cells, determining mitochondrial damage with an evident trigger of apoptotic signals. Such a mechanism of toxicity seems to be dependent by the redox state of methionine-35.

Keywords: Amyloid β-peptide; Aβ(31–35) fragment; Aβ(25–35) fragment; Methionine oxidation; Mitochondria; Apoptosis; Neurotoxicity

1. Introduction

Alzheimer's disease (AD) is one of the most common form of senile dementia, whose pathogenetic basis is related to the presence of amyloid β-peptide (Aβ), a β-sheet peptide formed by 39–43-amino acid residues, that aggregates in the brain to form the major component of characteristic deposits known as senile plaques [1,2]. X-ray diffraction data have shown that the conformation of Aβ is characterized by an antiparallel cross-β pleated sheet [3], although more recent solid state NMR evidence suggests that the peptide has a parallel β-sheet structure [4]. Nevertheless, aggregation occurs because of hydrogen bonding between β-strands, and the resulting fibrils have axes perpendicular to the β-strand and parallel to the cross-linking hydrogen bonds [3].

Among the Aβ fragments studied so far, the Aβ(25–35) peptide i.e. GSNKGAIIGLM, represents the shortest fragment of Aβ, processed in vivo by brain proteases [5]. Therefore, this peptide exhibits significant levels of molecular aggregation, retaining the toxicity of the full-length peptide, although it is lacking of metal binding sites. In line with this finding, it has been proposed that Aβ(25–35) peptide represents the biologically active region of Aβ [6,7]. However, although the deposition of Aβ in the central nervous system is a hallmark of AD and a possible cause of neurodegeneration [1,8,9], several reports have suggested that some non-aggregated amyloid molecules and its peptide fragments, may intercalate into the plasma membrane and directly alter membrane activities [10,11]. Recent studies evidenced that at earlier stages of AD, the non-aggregated form of Aβ fragments namely Aβ(25–35) mono/oligomer forms are also able to cross the cellular plasmatic membranes inducing intracellular mechanisms of toxicity [12,13]. Previous papers have reported that programmed cell death pathways may be involved in the mechanisms responsible for AD [14,15]. On the basis of these findings, it seems particularly interesting to further investigate the mechanism of toxicity induced by the non-aggregated forms of Aβ(25–35), trying to evidence the existence of apoptotic events in the toxic mechanism mediated by Aβ(25–35).

Critical events in apoptosis are the mitochondrial swelling accompanied by the disruption of membrane potential and the release of cytochrome *c* from the mitochondrial inter-membrane space. The released cytochrome *c* molecules, together with cytosolic factors, appear to be involved in the activation of caspase family proteases, a process which is upstream of DNA fragmentation and other apoptotic events [16,17].

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Abbreviations: Aβ, amyloid beta-peptide; AD, Alzheimer's disease; DMSO, dimethylsulfoxide; FITC, fluorescein isothiocyanate; MTS, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PTP, mitochondrial permeability transition pore; $\Delta\Psi_m$, mitochondrial transmembrane potential

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Moreover, in this study a comparison was also performed concerning the apoptotic signals caused by Aβ(25–35) peptide with respect to those showed by the Aβ(31–35) fragment. On the other hand, it is known that this Aβ derived pentapeptide, i.e. IIGLM, although not exhibiting aggregation phenomena, is able to determine a large number of toxic effects, including the activation of apoptotic pathways in cultured cortical neurons [18].

Another important feature characterizing the two examined fragments is the presence of methionine in the C terminal position; it is well known that the oxidation state of this residue strongly modifies the properties of Aβ [19]. In fact, it has been reported that oxidation of methionine significantly impairs the rate of amyloid formation, alters the fibril morphology and modifies the neurotoxic properties of beta amyloid [20]. Elevated levels of oxidized Aβ peptides were found in AD brains [21]. However, although the exact mechanism responsible for the Aβ-mediated toxicity still remains unclear, the methionine-35 side chain of Aβ is thought to play a critical role in this process, because it has been clearly shown that methionine-35 is the residue in Aβ most susceptible to oxidation in vivo [22].

In this study, we show that both Aβ(31–35) and Aβ(25–35) peptides induce apoptotic effects on isolated brain mitochondria and the redox state of methionine-35, mainly observable in the Aβ(31–35) peptide, play a key role in the induction of programmed cellular death pathways and toxic events. The obtained results appear to be of particular interest and cast light on new biochemical aspects which could be connected to the complex mechanism of AD pathogenesis.

2. Materials and methods

2.1. Preparation of peptides

Aβ(31–35) and Aβ(25–35) peptides with methionine, which is either in the oxidized and reduced form or substituted with norleucine (where —S group of methionine is replaced by a $—CH_2$) were synthesized and purchased from Peptide Specialty Laboratories GmbH (Heidelberg, Germany). Peptides were dissolved in dimethylsulfoxide (DMSO) at a concentration of 2.5 mM and stored at −80 °C. In previous studies [7,23] these conditions have been shown to lead to the predominance of the soluble monomeric form of these peptides. In any case, in order to verify the non-aggregated form of the peptides, quantitative measurement of Congo red (from Sigma) binding was carried out as described by Wood et al. [24].

In all control experiments, the concentration of DMSO (i.e., <0.5%) was the same as that present in the peptide solutions.

2.2. Cell line

PC12 cells (a clonal line of rat pheochromocytoma) were cultured in a 5% CO_2 atmosphere at 37 °C in RPMI with HEPES 10 mM, glucose 1.0 g/l, $NaHCO_3$ 3.7 g/l, penicillin 100 units/ml, streptomycin 100 μg/ml, 10% fetal calf serum, and 15% horse serum.

2.3. In vitro cytotoxicity assay

Cell viability was determined by a modified MTS [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Cell Titer 96; Promega Corporation, Madison, USA), which is based on the conversion of tetrazolium salt by mitochondrial dehydrogenase to a formazan product spectrophotometrically measurable at 490 nm. PC12 cells were plated in 96-well plates at a density of 10 000 cells/well and maintained for 16 h in complete medium [25].

Cells were then incubated in the absence (control) and presence of 40 μM Aβ(31–35) and Aβ(25–35) with reduced, oxidized and norleucine-substituted methionine-35 staurosporine 10 μM was used as positive control of 100% of cellular death (data not shown). After 48 h of peptide-incubation, 20 μl of MTS reagent (2.0 mg/ml) was added to each well. The cells were then incubated for 30–45 min at 37 °C in a 5% CO_2 incubator. The reaction was stopped by adding 25 μl of 10% SDS. The plates were read with a microplate reader (SpectraCount, Packard Bioscience Company, Groningen, Netherlands) at 490 nm. Each data point was obtained using a triplet-well assay.

2.4. FITC labeling of peptides

Fluorescein isothiocyanate (FITC) was purchased from Sigma. Labeling was conducted according to the manufacturer's recommendations. Briefly, FITC was freshly dissolved in DMSO to 1 mg/ml, and added to 2 mg/ml of each peptide in 50 mM potassium phosphate buffer (final pH 7.6) to a final concentration of 25 μg/ml. The calculated molar ratio of FITC to peptides was about 1:10. After incubation for 16 h in the dark at 4 °C, 50 mM NH_4Cl was added to inactivate the residual FITC. The solutions were left in the dark for an additional 2 h at +4 °C, and stored in aliquots at −20 °C.

At the moment of experiments fluorescent compounds were added to PC12 cell suspensions to a final concentration of 40 μM. Incubation has been performed for 2 h at +4 °C and +37 °C. Thereafter, cells were washed three times with saline solution (PBS) and observed at a confocal fluorescence microscope (Olympus IX-70 System). Images have been taken by using a 40× objective. Processing of images was carried out using the software package Photoshop (Adobe Systems Inc., Mountain View, CA, USA).

2.5. Mitochondria preparation

Non-synaptic brain mitochondria were isolated from male Sprague–Dawley rats weighting approximately 250–300 g, according to the literature [26]. Briefly, the cerebral hemispheres were homogenized in ice-cold buffer (280 mM sucrose, 1 mM EDTA, and 10 mM HEPES, pH 7.4). The homogenate was centrifuged at $1000 \times g$ for 10 min to remove cell debris. The resulting supernatant was centrifuged at $10\,000 \times g$ for 15 min to isolate the mitochondrial pellet. Finally, the mitochondria were purified from pellet on a Ficoll gradient and suspended in the homogenization buffer. Protein concentrations were measured by using the Bradford assay kit from Bio-Rad (BioRad Laboratories, Hercules, CA).

2.6. Analysis of mitochondrial oxygen consumption

For studying mitochondrial respiration, 1 mg of mitochondrial protein/ml was incubated in 280 mM sucrose, 10 mM HEPES, 1 mM EDTA, 10 mM KCl, pH 7.4 in the presence of 40 μM peptides under analysis for 30 min at 37 °C. Respiration rates were measured using substrates that enter the electron transport chain selectively at the following specific complexes: for complex I, glutamate (1.7 mM) and malate (1.7 mM); for complex II, succinate (2.5 mM) with NADH dehydrogenase inhibitor (2 μM rotenone). Oxygen consumption was measured at 37 °C with a Clark-type oxygen electrode (Strathkelvin Instr., Glasgow, UK) under continuous stirring.

2.7. Mitochondrial swelling

Swelling of isolated rat brain mitochondria was estimated spectrophotometrically as a decrease in absorbance measured at 540 nm. Mitochondria (0.5 mg protein/ml) were suspended in 280 mM sucrose, 10 mM HEPES, 2.5 mM KH_2PO_4, pH 7.4, and pre-incubated in a thermostated cuvette (37 °C) in the absence (control) and presence of 40 μM of Aβ(31–35) and Aβ(25–35) peptides with oxidized, reduced and norleucine-substituted methionine. Mitochondrial swelling was triggered by the addition of energizing substrates (2.5 mM for succinate in the presence of 2 μM rotenone and 1.7 mM glutamate plus 1.7 mM of malate). Mitochondrial swelling was also assessed both in the presence of 50 μM $CaCl_2$ as maximum inducer of PTP (data not shown) and of 1 μM cyclosporin A (CsA) an inhibitor of PTP.

2.8. Detection of cytochrome c release

Freshly isolated mitochondria were incubated in 280 mM sucrose, 10 mM HEPES, 1 mM EDTA, pH 7.4, at 37 °C for 30 min in the absence (control) and presence of 40 μM of Aβ(31–35) and Aβ(25–35) peptides with oxidized, reduced and norleucine-substituted methionine. The peptide concentration was set at 40 μM, as suggested by our preliminary observations [27,28] and by previous reports [29].

After the incubation time, mitochondria were spun at 15000 × *g* for 5 min at 4 °C. The resulting pellet and supernatant fractions were used for the detection of the cytochrome *c* by Western blotting analysis. Supernatants and pellets proteins were separated by 14% SDS–PAGE, blotted onto a nitrocellulose membrane, probed by the anti cytochrome *c* mAb (clone 7H8.2C12, PharMingen, San Diego, California) and developed by an amplified detection method (Opti-4CN, BioRad Laboratories, Hercules, CA).

2.9. Statistical analysis

All of the data were expressed as means ± S.E. of five to seven independent experiments. Data were analyzed for statistical differences by one-way analysis of variance (ANOVA) as well as by the two-tailed Student's *t* test; a *P* value of less than 0.05 was considered significant.

3. Results

All Aβ peptides employed in our experimental procedures were in non-aggregated form i.e. predominately monomeric, in accord with previous reports [7,23,30].

The first point investigated in this study was to clarify the role played by methionine-35 in the mechanism of Aβ induced cellular toxicity. Thus, the toxic effects of Aβ(31–35) and Aβ(25–35) peptides, where the C-terminal methionine was substituted by norleucine or present in the reduced and in the oxidized form, were evaluated on PC12 cells (a cellular line of rat pheochromocytoma). PC12 cell viability was measured by the MTS method, which is routinely used to evaluate the number of viable cells in proliferation. Fig. 1 shows the cellular survival, following the exposure to different Aβ fragments. It appears evident that after 48 hours of incubation, Aβ(31–35) and Aβ(25–35) peptides bring about a larger toxic effect on viability of PC12 cells than that shown by Aβ(31–35)Met → Nle and Aβ(25–35)Met → Nle treatment, which do not induce a significant cellular death with respect to the control. It should also be noted that the major extent of cellular death (22% with respect to the control) is induced by Aβ(31–35) peptide where methionine-35 is in the reduced form.

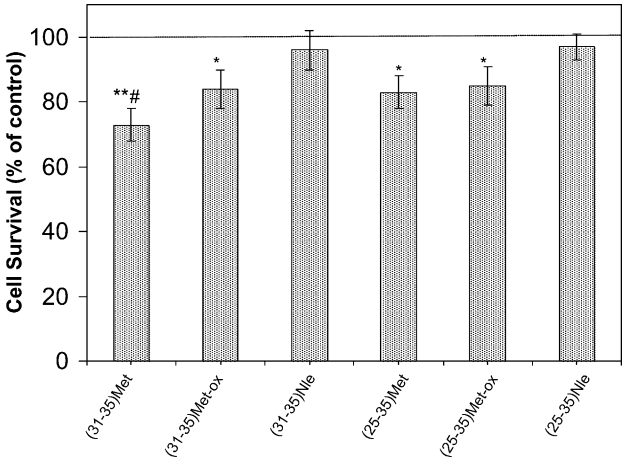

Fig. 1. Effects of 40 μM Aβ(31–35) and Aβ(25–35) peptides with reduced ((31–35)Met; AβP(25–35)Met), oxidized ((31–35)Met-ox; (25–35)Met-ox) and norleucine-substituted ((31–35)Nle; (25–35)Nle) methionine on PC12 cell survival, expressed as percent of cells untreated (control line = 100%). Cells (10000 cells/well) were cultured with peptides under analysis (experimental conditions are reported in Section 2), and the viability of PC12 cells was measured by MTS assay after 48 h of incubation. All values indicate means ± S.E. of eight independent experiments. Significantly different from controls: $^{*}P < 0.05$; $^{**}P < 0.01$; Significantly different from other peptides: $^{\#}P < 0.05$.

The next point investigated was to clarify the cellular compartment involved in the toxic mechanism induced by these two short fragments of native Aβ peptide. Hence, the capacity of PC12 cells to internalize FITC labelled Aβ peptides was investigated. It is quite evident (see Fig. 2) that both Aβ(31–35) (panel A) and Aβ(25–35) (panel B) peptides have the ability to cross the plasmatic membranes after two hours of incubation at 37 °C, being detectable into the cytoplasm and nucleus. The process of internalization observed in the experiments performed at 37° but not at 4 °C (data not shown), allow us to hypothesize the involvement of a capping mechanism in the entire process of internalization of Aβ peptides. No differences for the ability to enter the cells were found among the Aβ peptides where methionine-35 was in the reduced and oxidized form or substituted by norleucine (data not shown).

Although, there are no direct evidences yet, we have postulated that both Aβ(31–35) and Aβ(25–35) peptides may directly interact with mitochondria within the PC12 cells; hence we determined, as index of mitochondrial functionality, the oxygen consumption and the mitochondrial swelling in isolated brain mitochondria treated with Aβ(31–35) and Aβ(25–35) peptides. Fig. 3 shows the respiratory activity of isolated brain mitochondria in the presence of two distinct substrates, namely succinate (with rotenone) and glutamate plus malate. It is evident that the addition of 40 μM of Aβ(31–35) and

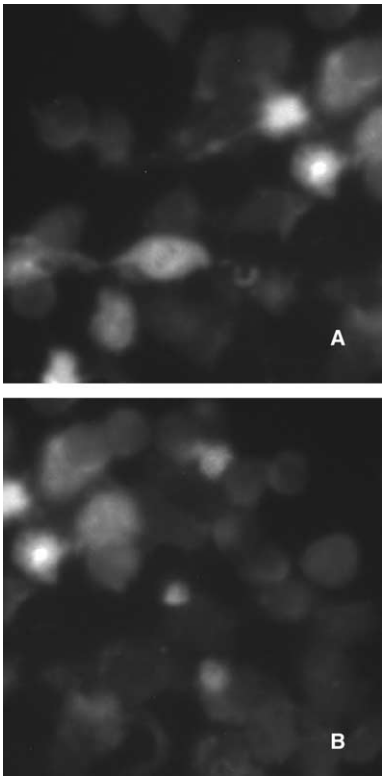

Fig. 2. Confocal fluorescence microscopy images of PC12 cells labelled with FITC-Aβ(31–35) (panel A) and FITC-Aβ(25–35) (panel B) peptides. Experimental conditions are reported in Section 2.

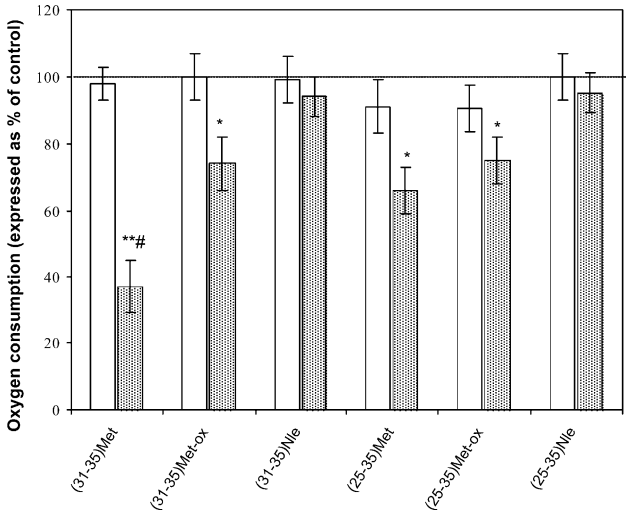

Fig. 3. Effect of AβP(31–35) and AβP(25–35) peptides on mitochondrial oxygen consumption (expressed as % of control line = 100%) in the presence of succinate (white bars) and glutamate/malate (grey bars) as substrates. Measurement of respiration rate was taken at 4th minute. Experimental conditions are reported in Section 2. Absolute values of oxygen consumption in the absence of peptides were 30.0 ± 1.8 nmol/min/mg protein ($N = 8$) with glutamate/malate and 50.14 ± 3.0 nmol/min/mg protein in the presence of succinate ($N = 8$). Values presented are means ± S.E. obtained for eight independent experiments. The statistical significance was determined as reported in Section 2. ($^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. other peptides).

Aβ(25–35) peptides to mitochondria does not significantly modify succinate oxidation when compared to that measured in control experiment, whereas the glutamate/malate oxidation was significantly reduced by the presence of Aβ peptides, unlike in mitochondria treated with Aβ fragments containing norleucine residue at position 35. Besides, it should be noted that Aβ peptides with methionine in reduced state, and in particular the Aβ(31–35), showed a larger effect, as compared to their analogous with methionine in the oxidized form, inducing

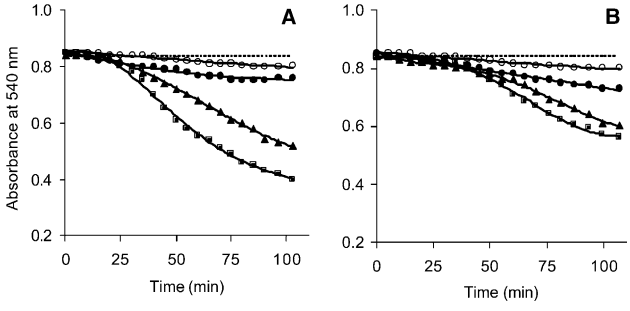

Fig. 4. Effect of Aβ(31–35) (panel A) and Aβ(25–35) (panel B) peptides on mitochondrial swelling. Swelling was induced by the addition of glutamate/malate in mitochondria treated with 40 μM peptide with reduced (■), oxidized (▲) and norleucine substituted (●) methionine. The mitochondria untreated represent the controls (○). Dashed lines represents data obtained with 40 μM of all peptides in the presence of 1 μM CsA. The experiments shown are representative of eight separate measurements. Experimental conditions are reported in Section 2.

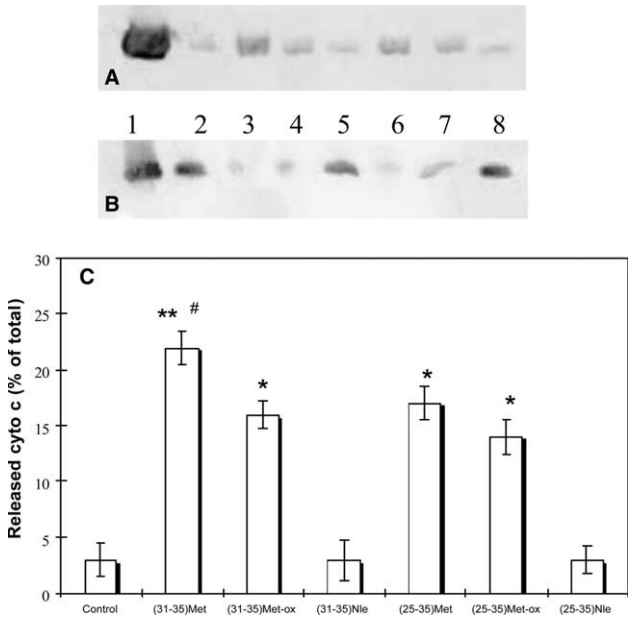

Fig. 5. Cytochrome *c* release from brain purified mitochondria untreated (control: lane 2) and after incubation with 40 μM (31–35)Met (lane 3), (31–35)Met-ox (lane 4), (31–35)Nle (lane 5), (25–35)Met (lane 6), (25–35)Met-ox (lane 7) and (25–35)Nle (lane 8). Besides, in lane 1: standard commercial horse heart cytochrome *c*. (A) Representative Western blotting of the released cytochrome *c*. (B) Representative Western blotting of remained cytochrome *c* in the mitochondrial pellets. (C) Cytochrome *c* release expressed as percent of total (released cyt + cyt remained in mitochondrial pellet). The data were obtained from Western blotting/densitometry. Results shown are means ± S.E. obtained for eight separate preparations ($^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. other peptides).

a respiration inhibition of ~56% and 30% (with respect to the controls) for Aβ(31–35) and Aβ(25–35) peptides, respectively.

Afterwards, rat brain mitochondria incubated with peptides and energized with glutamate/malate were monitored at 540 nm, as reported in Section 2 (see Fig. 4) in order to show the ability of Aβ(31–35) (panel A) and Aβ(25–35) (panel B) in inducing mitochondrial swelling.

It is evident that all Aβ peptides tested, and mainly the Aβ(31–35) with methionine in the reduced state, have the ability to induce mitochondrial swelling. Since these effects were completely prevented by CsA, a well-known inhibitor of PTP, our results indicate that both Aβ(31–35) and Aβ(25–35) peptides bring about an opening of the mitochondrial megapore leading to the induction of PT.

Another critical event in the apoptotic pathway is represented by the release of cytochrome *c* from the mitochondrial inter-membrane space. Thus, the amount of cytochrome *c* released from isolated rat brain mitochondria (1 mg/ml of proteins), following the exposure to 40 μM of Aβ(31–35) and Aβ(25–35) peptides at 37 °C for 30 min, was determined. After incubation, supernatant and pellet fractions were collected and subjected to immunoblotting analysis (see Fig. 5A and B). It is evident from the densitometric analysis of the bands (Fig. 5C) that in mitochondria treated with Aβ(31–35) and Aβ(25–35) peptides, the release of cytochrome *c* is more evident with respect to that observed in control experiment (mitochondria untreated) and in mitochondria treated with Aβ(31–35)Nle and Aβ(25–35)Nle peptides.

4. Discussion

A number of studies have recently shown that soluble forms of Aβ might contribute to AD dysfunctions and pathophysiology by altering neuronal functions by means of other mechanisms than plaque formation. The results presented here show that Aβ(31–35) and Aβ(25–35) peptides, in their non-aggregated form, i.e. predominantly monomeric [7,23,31], induce neurotoxicity via an apoptotic pathway, mediated by mitochondria.

Firstly, it has been demonstrated that these two short Aβ fragments have the ability to enter within the cells. This finding supports previous reports providing evidences that Aβ(31–35) and the soluble forms both of Aβ(25–35) and Aβ(1–40) peptides of beta amyloid, unlike their aggregated form, compete for binding to serine proteinase inhibitor (serpin)-enzyme complex receptor (SEC-R), a receptor for α1-antitrypsin and other serine protease inhibitors [23]. At this regard, it has been formulated the hypothesis of the existence of a protective cell mechanism, coming into play at the earlier stages of the AD and removing the extracellular Aβ fragments still in the monomeric and soluble form. On the contrary, our data indicate that the monomeric short Aβ(31–35) and Aβ(25–35) peptides, after their internalization within the cells, induce mitochondrial damage that leads to cell death by an apoptotic pathway.

As a matter of fact, several apoptotic signals, such as inhibition of mitochondrial respiration, induction of mitochondrial swelling and release of cytochrome *c*, occur in isolated brain mitochondria following peptides exposure. Aβ(31–35) and Aβ(25–35) interact with the mitochondrial inner membrane interfering probably with some dehydrogenases producing NADH and/or at complex I of the mitochondrial respiratory chain and inducing mitochondrial swelling. Previously, Canevari et al. [32] showed that Aβ(25–35) inhibited mitochondrial respiration at level of complex IV. It is likely that this discrepancy originate from the sampling procedure utilized in these studies. In particular, it should be noted that, differently to our experimental conditions, Canevari et al. [32] utilized Aβ(25–35) peptide in a predominantly aggregated form.

Mitochondrial swelling may be due to the opening of the mitochondrial permeability transition pore (PTP), a poly-protein complex formed at the contact sites between the inner and the outer mitochondrial membranes. The formation of PTP results in a loss of the mitochondrial transmembrane potential, $\Delta\Psi_m$. Because $\Delta\Psi_m$ reduction represents an additional event linked to apoptotic processes, this finding further supports our hypothesis, concerning the involvement of an apoptotic pathway in the mechanism responsible for Aβ(31–25) and Aβ(25–35)-mediated mechanisms of toxicity [33]. Whereas the nature of the mitochondrial membrane conformational change leading to PTP is still unclear, it is well known that it may be induced by large amounts of Ca^{2+}, as well as by a variety of agents or conditions determining oxidation or cross-linking of membrane protein thiol groups [34]. On the basis of these reports and of the results obtained in our experiments performed in the presence of the Aβ peptides containing at C-terminal norleucine, it is evident that methionine-35 residue in Aβ peptides might play a key role in PTP induction. In this respect, our observation outlines the major toxic effects exerted by Aβ peptides, in particular Aβ(31–35), with methionine in reduced state, which can be discussed on the light of recent reports displaying that also any reducing effectors, (i.e. curcumin, reducing Fe^{3+} into Fe^{2+}, which in turn reacts with H_2O_2) can increase the rate of $HO^{\cdot}$ production. This highly reactive radical may oxidize critical thiol groups, leading to PTP opening and to other mitochondrial dysfunctions [35]. In the same way, Aβ(31–35) and Aβ(25–35) peptides could act as reducing agents, being able to modify the oxidative state of the iron and to interfere with the mitochondrial membrane integrity.

The rupture of the outer membrane, as a consequence of mitochondrial swelling, may bring about a release of cytochrome *c* and other proteins in the inter-membrane space which, in turn, triggers the caspase cascade by activating pro-caspase-3. In line with the reported apoptotic pathway, in this study it has been shown that both Aβ(31–35) and Aβ(25–35) peptides induce a significant release of cytochrome *c*. However, the mechanism by which soluble Aβ(31–35) and Aβ(25–35) peptides containing methionine in the reduced state, damage to a different extent cell cultures and isolated mitochondria is not well clarified yet. Previous studies have yet suggested the possibility that Aβ with different length may have distinct mechanisms of toxicity [36,37]. Some differences could be ascribed to the unique sequence of amino acids relative to methionine. At this regard, it has been suggested that the interaction of isoleucine (residue 31 and 32) with the —S atom of Met-35, may be the critical factor in Aβ peptide-induced toxicity [38,39]. Thus, it is likely that the presence of isoleucine 31 at the —NH2 terminal and the short length of the Aβ(31–35) peptide might explain the different effects induced by Aβ(25–35) with respect to that observed with Aβ(31–35) peptide. Furthermore, we provide evidence that although oxidation of Met-35 to Met-35^{OX} does not affect the internalization process of the Aβ(31–35) peptide, it significantly affect the amplitude of the Aβ(31–35) mediated toxic effects. This finding might result from differences in the ability to interact with lipid bilayer of the membranes. Indeed it is known that oxidation of methionine-35 residue modifies the solubility of the amyloid peptides [40,41], limiting probably their ability to interact with mitochondrial membrane and most likely their toxicity. Thus it is suggestive to hypothesize that a decreased or inhibited activity of methionine sulfoxide reductase, as found in AD brains [42], may be part of a cell protective mechanism useful to not reduce back methionine-35 residue and to maintain Aβ peptide no longer neurotoxic.

In summary, our results support the possibility that the soluble forms of Aβ peptide, that precede the formation of mature amyloid aggregates, may induce neurotoxicity via an apoptotic pathway. Such mechanism seems to be modulated by the redox state of methionine-35.

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