Vol. 205, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1994 Pages 120-126

CONFORMATIONAL MAPPING OF AMYLOID PEPTIDES FROM THE PUTATIVE NEUROTOXIC 25-35 REGION

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Received September 14, 1994

The secondary structure of amyloid βA(25-35) and its deletion analogues was studied by circular dichroism (CD), Fourier transform infrared (FTIR) spectroscopy and molecular dynamics calculation. Data of our comparative CD and FTIR measurements in trifluoroethanol suggest that βA(25-35)NH_2 has a preferred β-sheet conformation. Contrary to this βA(31-35)NH_2 tends to adopt a β-turn conformation. Based on the comparable neurotoxic effect of βA(25-35)NH_2 and βA(31-35)NH_2 the neurotoxicity likely involves the same 31-35 core sequence and the "biologically active conformation" is a β-turn rather than a β-sheet structure.

Deposition of β-amyloid protein (βA) in the core region of senile plaques is a key feature of Alzheimer disease (AD). ßA is a small polypeptide fragment of approximately 39 to 43 amino acids which is generated by proteolytic cleavage of a large membrane spanning protein, the β-amyloid precursor protein (1,2). In rat nerve cell culture ßA(1-40) has been reported to have both neurotrophic and neurotoxic effects depending on neuronal age and the concentration of the peptide (3). Both properties were resided in residues 25-35 of ßA, a region showing homology with the sequence of tachykinin neuropeptides (4). It was also demonstrated that tachykinins can reverse the effect of ßA(1-40) and ßA(25-35) (5). Substance P (SP) proved to be a very potent inhibitor of the action amyloid fragments which suggests the involvement of SP receptors in amyloid recognition. However, ßA peptides were not found to interact with tachykinin receptors (6,7)

It has also been reported that amyloid peptides alone have no effect on human cortical neuronal survival but render the neurons more vulnerable to Ca^{2+}-influx and excitatory amino acids (glutamate, N-methyl-D-aspartate etc) neurotoxicity (8).

One of the factors which has an influence on the in vitro biological activity of amyloid peptides is that they may adopt different conformations depending on solvent, pH, ionic strength etc. ßA peptides are weakly soluble in physiological salt solutions, a property

0006-291X/94 $5.00

that likely contributes to their ability to form β-sheet aggregates characterictic of plaques (9,10).

The low concentration (1-100 nM) of amyloid peptides applied in culture experiments indicates that they act via high affinity receptors (3). It can be assumed that an "active segment" of amyloid, located within residues 25-35, is responsible for receptor binding. To find a correlation between neurotoxicity and conformation, this study is aimed at determining the secondary structure of βA(25-35) and its N-terminal deletion analogues. Conformational studies were performed by circular dichroism (CD) and Fourier-transform infrared (FTIR) spectroscopy and molecular dynamics (MD) calculations. Previous studies have shown that the pentapeptide (31-35) has the same neurotoxicity as βA42 (11).

MATERIALS AND METHODS

βA(25-35) (GSNKGAIIGLM) and its deletion fragments were synthesized with amide at the C-terminus by the solid phase technique utilizing Boc chemistry. ßA(25-35) and ßA(31-35) fragments were also prepared in free carboxyl form. Side chain protecting groups were as follows: Bzl(Ser), 2-ClZ(Lys). The peptide chains were elongated on MBHA resin (0.6-0.8 mmol/g) and the syntheses were carried out manually. Couplings were performed with dicyclohexylcarbodiimide (DCC) with the exception of Asn which was incorporated in HOBt-ester form. The Boc group was removed by treatment with 50% trifluoroacetic acid (TFA) in CH_2Cl_2. After completing the synthesis, the peptide was cleaved from the resin with liquid HF. The free peptides were solubilized in 95% TFA, filtered and lyophilized. The crude peptides were purified by reverse-phase HPLC using a BST-SI-100S $10C_{18}$ column. The purity was checked by RP-HPLC on a Nucleosil 5 C_{18} column. Amino acid analysis resulted in the expected amino acid composition.

Circular dichroism spectra were measured on a Jobin-Yvon Mark VI dichrograph. Measurements were carried out in 0.02 cm cells. Trifluoroethanol, TFE (NMR grade, Aldrich) and double distilled water were used as solvents. The concentration of the samples was 0.5 mg/ml. Mean residue ellipticity, $[\Theta]_{MR}$, is given in deg.cm^2/dmol.

Infrared measurements (at a resolution of 4 cm^{-1}) were performed at room temperature on a Nicolet Magna 750 spectrometer. Peptide solution ($c \approx 1$ mg/ml) were prepared in TFE. The IR spectrum of TFE (containing traces of water) was obtained under identical conditions. The deformation vibrational band of water between 1700-1600 cm^{-1} was removed by substracting the spectrum of water in TFE on the basis of the combination band of OH stretching and HOH deformation at 5293 cm^{-1}. The spectral contribution of trifluoroacetate at about 1675 cm^{-1} was removed on the basis of the intensity of CF_3 ν_{as} and ν_s vibrations measured in the KBr spectra of the peptides and the CF_3COONa, respectively. KBr cells of 0.041 cm were used. The spectra were analyzed by a normalized, least-squares curve-fitting program, using products of Gauss and Lorentz curves (Holly et al., unpublished). The selection of component curves was assisted by the Fourier self-deconvolution method of Mantsch et al. (12).

Molecular dynamics energy minimizations and dinamics trajectory analyses were performed using INSIGHT-II (Biosym Technologies Inc) on an IBM RS/6000 320/H computer. The conformational energy was minimized first with backbone torsional constraints, then fully relaxed. Using distance depending dielectric constant, molecular simulation was obtained by heating and equilibration (0→1600 K in 10 ps), and simulation steps (1600 K, 500 ps).

RESULTS AND DISCUSSION

CD measurements were performed in TFE and TFE/water mixtures. TFE is well known to stabilize H-bonded α-helical and ß-turn conformations even in small peptides.

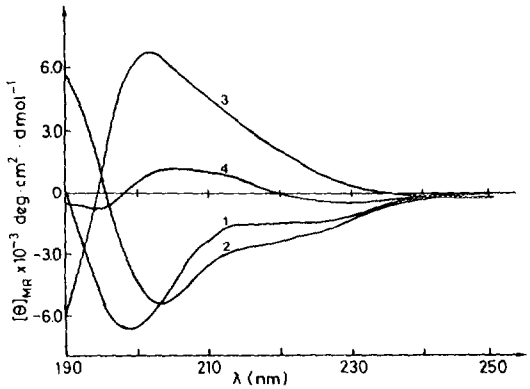

Fig. 1. Circular dichroism spectra of βA(25-35)OH (1), βA(25-35)NH_2 (2), βA(31-35)OH (3) and βA(31-35)NH_2 (4) in TFE. Peptide concentration: 0.5 mg/ml.

TFE has also been suggested to mimic the low dielectric constant environment of biological membranes (13) and to promote the adoption of conformations of peptides in receptor-bound state.

Conformational studies of βA(25-35). CD spectra of βA(25-35)NH_2 were recorded in water (pH 6) and TFE in the concentration range of 2-0.05 mg/ml. No concentration dependent change of the secondary structure was observed upon dilution of the stock solution (data not shown). Fig. 1 shows the CD spectra of βA(25-35)NH_2 and βA(25-35)OH in TFE. The low intensity blue shifted type-C spectrum (14) of βA(25-35)NH_2 is indicative of the presence of ordered, possibly 3_{10}-helix conformation. The CD spectrum of βA(25-35)OH differs significantly from that of the amide form. The introduction of a negative charge into the C-terminus results in a shift of the conformational equilibrium towards unordered conformation.

Fig. 2 illustrates the infrared spectrum of βA(25-35)NH_2 measured in TFE solution. The most characteristic feature of the spectrum is the intense amide I band at 1626 cm^{-1}, which accompanied by a weaker component band at 1688 cm^{-1}, identifies antiparallel β-sheet conformation (15). The component band at 1662 cm^{-1} indicates the presence of weakly H-bonded (solvated) α-helix or 3_{10}-helix (repeats of type III β-turns), while the band at 1676 cm^{-1} belongs to buried amide carbonyls. The component band at 1646.5 cm^{-1} is characteristic of aperiodic (unordered) conformation. Thus, βA(25-35)NH_2 is present as a mixture of three conformers: helix, β-sheet and unordered in TFE.

Based on recent CD studies by Terzi et al. (16), βA(25-35)OH in buffer solution at pH 7.4 forms antiparallel β-sheet , independent of peptide concentration. Contrary to this, βA(25-35)NH_2 in water was found to show CD spectra characteristic of predominantly random conformation (16). Our FTIR studies on the same amide fragment (Fig. 2) revealed a significant amount of β-sheet conformation even in TFE known to promote α-helix rather than β-sheet.

Conformational studies of deletion fragments. βA(26-35)NH_2 (not shown) and βA(27-35)NH_2 exhibit CD spectra of similar shape (type-C) but higher band intensities

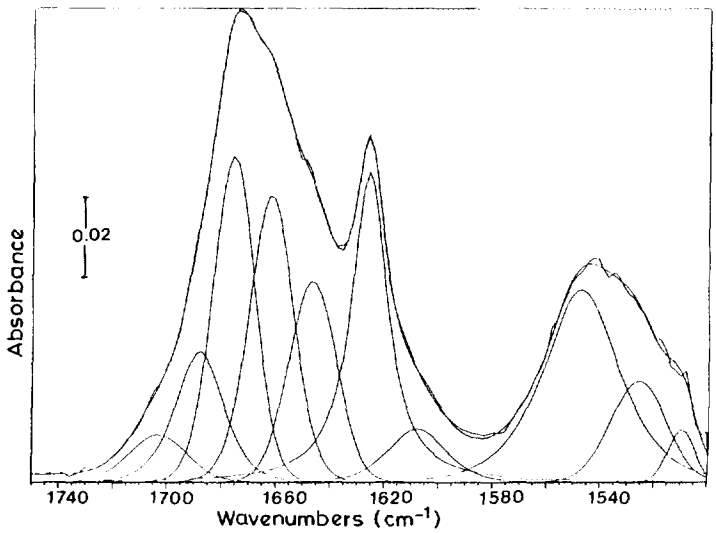

Fig. 2. FTIR spectrum of βA(25-35)NH_2 in TFE. The spectral contribution of trifluoroacetate has been substracted as described in Materials and Methods.

compared to that of βA(25-35)NH_2 (Fig. 3). Possibly the loss of the amino-terminal Gly and Ser residues results in the stabilization of the ordered conformation. The blue shifted negative ππ* band (199 nm) and the absence of a negative nπ* band in the spectrum of βA(28-35)NH_2 clearly indicate the predominance of unordered conformation of the octamer even in 100% TFE. The negative nπ* band shows up again in the spectra of βA(29-35) (not shown) and βA(30-35) reflecting an increasing amount of ordered conformers. The shape of the CD spectrum of pentapeptide ßA(31-35)NH_2 differs significantly from that of the hexa- and heptamers while the spectrum of tetrapeptide (32-35) resembles the hexapeptide again.

Conformation of βA(31-35). CD spectra of βA(31-35)OH and βA(31-35)NH_2 are shown in Fig. 1. In the spectrum of amide form the appearance of a broad positive band between 200-220 nm and a weak negative band between 220-240 nm can be explained by the presence of more than one conformer population. Gly in the i+2 position is expected to result in a type II β-turn giving rise to a class B CD spectrum (negative band between 220-230 nm and a stronger positive one above 200 nm) (14.) The shape of the CD curve and the low band intensities may be due to the presence of substancial population of unordered conformer(s) with a negative band below 200 nm which is in overlapping with the positive band of the β-turn. βA(31-35)OH shows a class C' CD spectrum in TFE. Class C' spectra can be correlated with distorted type II β-turns. The intense positive band is indicative of an increased population of the folded conformation. The class B to class C' spectral shift and concomitant intensity increase are likely the result of a salt bridge between the oppositely charged NH_3^+ and COO^- groups of the molecule.

The FTIR spectrum of βA(31-35)NH_2 shown in Fig. 4 is marked by a shoulder near 1640 cm^{-1}. In the curve-fitted spectrum the corresponding component band appears at

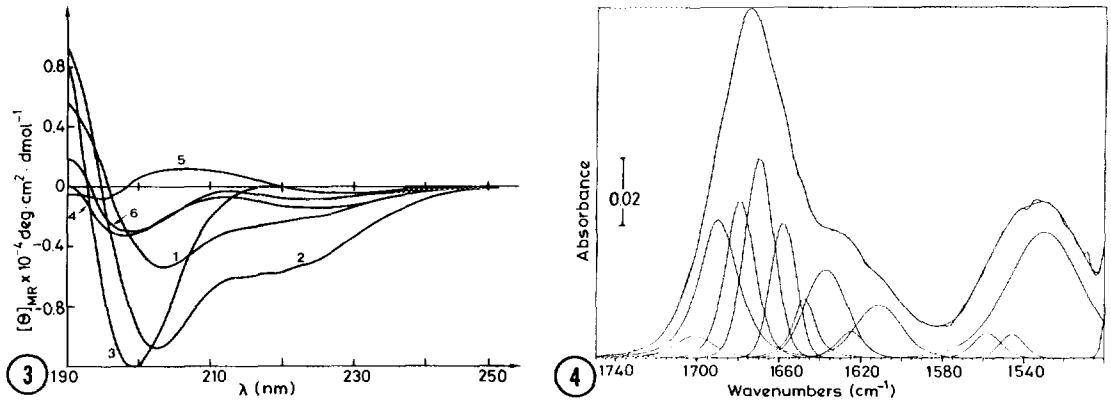

Fig. 3. CD spectra of deletion analogues of βA(25-35)NH_2. 25-35 (1), 27-35 (2), 28-35 (3), 30-35 (4), 31-35 (5), 32-35 (6) in TFE. Peptide concentration:0.5 mg/ml.

Fig. 4. FTIR spectrum of βA(31-35)NH_2 in TFE. The spectral contribution of trifluoroacetate has been substracted as described in Materials and Methods.

1637 cm^{-1}. This band can be correlated with the acceptor amide C=O of high population of H-bonded β-turns (17). Based on the infrared spectrum, this pentapeptide is present as a mixture aperiodic (1647.5 cm^{-1}), 3_{10}-helical (1658 cm^{-1}) and strongly associated conformers (1611 cm^{-1}). The component bands at 1669.5, 1679 and 1689.5 cm^{-1} likely reflect the presence of solvated and buried amide carbonyls.

Molecular dynamics calculations were performed on the 25-35 and 31-35 fragments with amide and carboxy C-terminal without applying any structural constraints. The computations are in essential agreement with the experimental data and suggest a multicomponent mixture of conformers with substantial population of β- and γ-turn structures.

CONCLUSION

CD spectroscopic mapping of the conformation of eight peptide fragments comprising residues 25-35 through 32-35 of βA revealed that it is the pentapeptide which features a unique folded conformation. Based on CD and FTIR studies supported by MD calculations, βA(31-35)NH_2 in TFE has a preferred H-bonded type II β-turn conformation likely stabilized by apolar interactions between the N-terminal Ile^1 and C-terminal Leu^4 or Met^5. In βA(31-35)OH the folded conformation is further stabilized by electrostatic attraction between the oppositely charged N- and C-termini.

A CD spectrum similar to that of amyloid pentapeptide was reported for pentapeptide Met-enkephaline when measured in TFE (18). Conformational energy calculation, in agreement with the CD data, predicted a type II' β-turn conformation for this peptide (19,20). The enkephaline antagonist Boc-Tyr-Pro-Gly-Phe-Leu-OH also shows class C' CD spectrum in TFE which has been suggested to reflect the adoption of a distorted type II β-turn (17).

The enkephaline peptides are thought to compete directly with opiates (morphine) for the brain opiate receptors, while having no primary structure similarities to opiates. The basis of the competition was suggested to lie in the similar secondary structure (19). The κ binding site which is the major opiate receptor class in human brain has been found to undergo marked changes in AD (21). Opioids can influence the release of acetylcholine, substance P and a number of other neurotransmitters that have been implicated in the pathogenesis of AD. For this reason it bears special interest to reveal the conformational analogy between the opiates and the neurotoxic amyloid fragments.

The amyloid and enkephaline pentapeptides are expected to assume various conformations in aqueous solution. However, at the receptor site a unique receptor-induced conformation may be present, which is possibly a β-turn. Like the similar biological activity of the different molecules morphine and enkephaline peptides lies in their similar steric structure, we may assume that the effective competition of amyloid fragments for tachykinin or other peptide hormone receptors is also based on similar secondary structure.

Our comparative CD and FTIR spectroscopic studies, in agreement with the CD data of Terzi et al. (16) strongly suggest that βA(25-35) has a preferred β-sheet conformation. Contrary to this, the pentapeptide βA(31-35)NH_2 tends to adopt a β-turn conformation in low-dielectric constant environment. The comparable neurotoxic effect of βA(25-35)NH_2 and βA(31-35)NH_2 suggests that neurotoxicity and binding to the tachykinin or other receptors involve the same 31-35 core sequence of amyloid and the "biologically active conformation" if required at all is a β-turn rather than a β-sheet structure.

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