The role of β-hairpins in aggregation of β-amyloid derived peptides

Elisabetta Galluppo[1], Claudia Honisch[1], Stefano Tartaggia[1], Mauro Carraro[2], Paolo Ruzza[1]

1. *Institute of Biomolecular Chemistry of CNR, Padova Unit, Padova, Italy,*
2. *Dept. of Chemical Sciences, University of Padua, Padova, Italy*

https://doi.org/10.17952/37EPS.2024.P1098

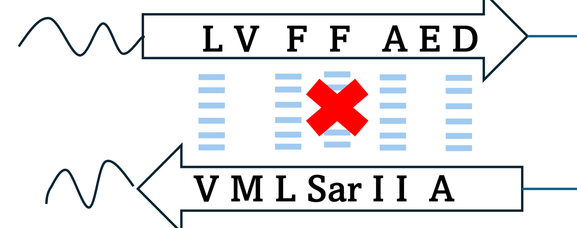
Alzheimer's disease (AD) is one of the most widely recognized and prevalent neurodegenerative conditions in today's society. However, despite its prevalence, effective pharmacological treatment is not currently available, leaving only palliative care options as the most helpful course of action. The pathogenesis of Alzheimer's disease (AD) involves the formation of proteinaceous plaques due to the aggregation of originally soluble oligomers of **amyloid-β peptides**[1]. These oligomers have been observed to adopt different conformations, including β-sheets, α-helices, and predominantly **β-hairpins**. β-hairpins are capable of assuming different configurations in space.

In order to investigate the mechanisms that govern β-hairpin orientation and develop an *in vitro* model of peptide aggregation for use in testing inhibitors of this process, two **Aβ17-36 derivatives** were synthesized; subsequently, their conformation was studied through circular dichroism spectroscopy (CD).

Synthesis

Fmoc-SPPS was conducted using a Wang resin preloaded with valine, employing HOBt/HBTU as the activator, while HATU was utilized exclusively for the couplings involving Ile[32] and D-Pro residues. To mitigate the propensity for β-sheet aggregation, an **N-Me-Gly (Sarcosine)** residue [2] was incorporated into both peptides, instead of a residue of glycine.

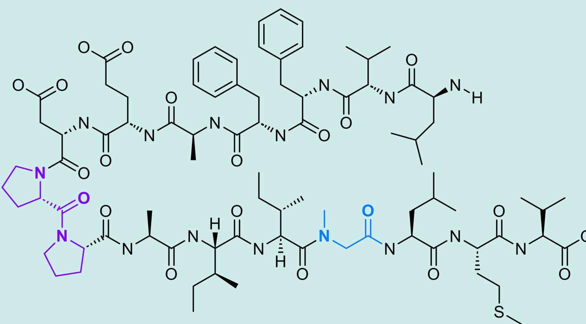
This residue modulates the hydrogen bonding pattern, promoting a *cis* conformation of the Ile-Sar peptide bond and disfavoring the aggregated form of the hairpin[3].



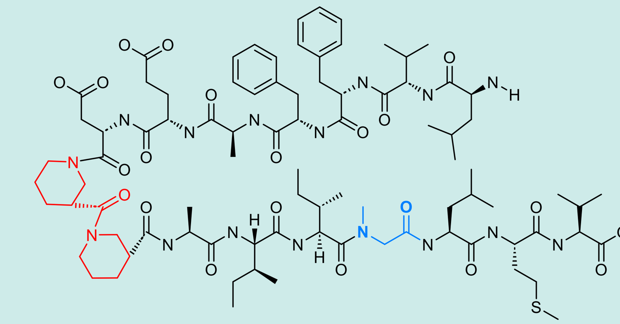
Purification

Purification of both peptides was carried out using reverse-phase HPLC on a C18 column with: (A) 0.05% TFA in H_2O and (B) 0.05% TFA in acetonitrile: H_2O 9:1 v/v. Linear binary gradient: 38-50 %B in 40 minutes. Due to the poor solubility of the peptides, to be eluted the samples are dissolved in a 9:1 v/v solution of acetonitrile: 10 mM ammonium acetate, pH 5.5.

*Sequence of **17-37pP** peptide:*
L[17]VFFAED[23]-pP-A[30]IISarLMV[36]



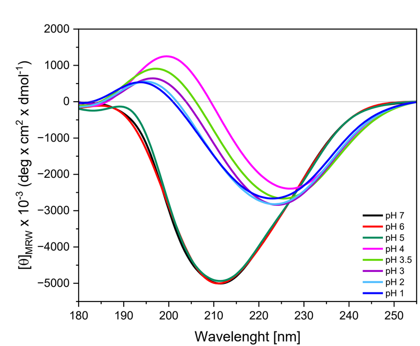
Compared to the original sequence Aβ17-36[2], we examined the conformation of two distinct loops: a sequential **homochiral (R)-(R-) dinipecotic acid (Nip)** segments[4] and the **D-Pro-L-Pro (pP)** loop[5].

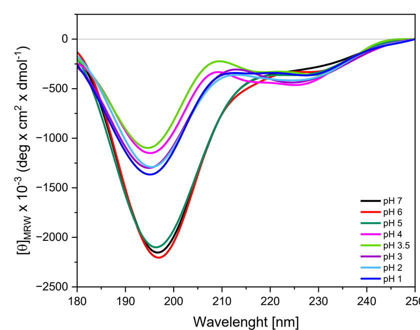


*Sequence of **17-37Nip** peptide:*
L[17]VFFAED[23]-Nip-Nip-A[30]IISarLMV[36]

Circular Dichroism

Effect of pH



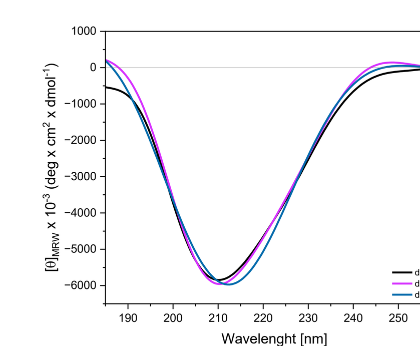


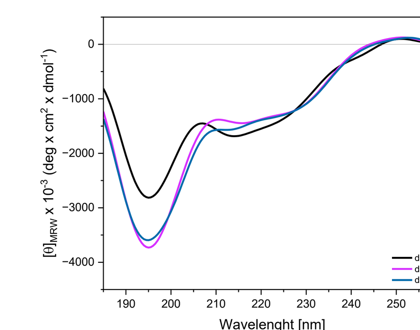
(up) 17-36pP (down) 17-36Nip *peptide **titration** with H_2SO_4 0.05 M. Peptide was dissolved in PB buffer 10 mM (0.1 mg/ml), cell pathlength 1 mm.*

The far-UV CD spectra of both *17-36pP* and *17-36Nip* peptides are found to be influenced by the pH values. In a 10 mM phosphate buffer (PB) with a pH of 7.4, both spectra exhibit a negative band within the 195-210 nm range, which is indicative of an unordered conformation. A pH value lower than 4 units results in the CD spectrum of the ***17-36pP*** peptide developing a negative band near 225 nm, the growth of a positive band at approximately 200 nm, and a negative band near 180 nm. This is indicative of a class B spectrum, according to the Woody classification[7], which suggests the presence of a **β-turn**. The intensity of these bands is influenced by the pH values, from pH 7.4 to 1.5.

The CD spectrum of the peptide ***17-36Nip*** at lowering pH values demonstrated a reduction in the intensity of the negative band at 198 nm, accompanied by a blue-shift at a low wavelength (195 nm), and the emergence of a negative band at approximately 225 nm. For this peptide, as well, the intensity of the bands was affected by pH values. The shape of this spectrum could not be attributed to any β-turn described thus far.

Effect of time

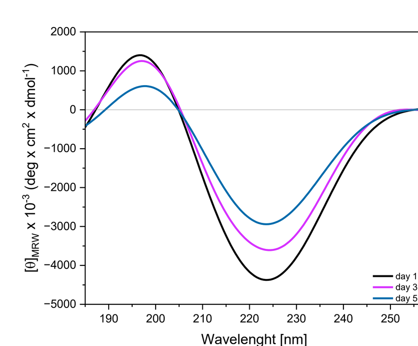


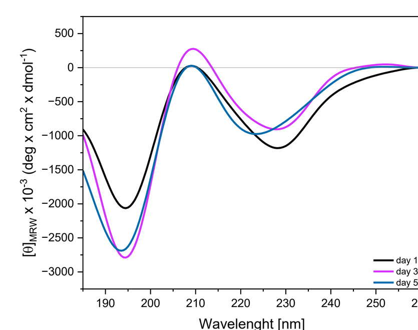


(up) 17-36pP (down) 17-36Nip *peptide dissolved in PB buffer 10 mM (0.5 mg/ml) **pH 7.4**, cell pathlength 1 mm, over **5 day**.*

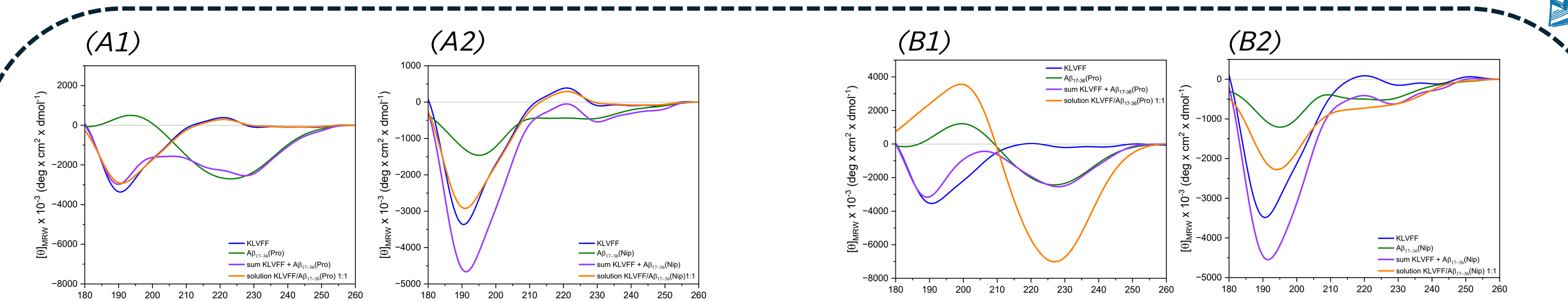
The ***17-36pP*** peptide exhibits no conformational change when incubated in PB buffer at pH 7.4 for five days at 25°C. However, at acidic pH conditions, the intensity of the CD bands of the peptide undergoes a change over time, indicating a tendency towards **aggregation**.

The pH 7.4 spectrum of the ***17-36Nip*** peptide is influenced by incubation at 25°C, resulting in an increase in the intensity of the negative band at approximately 190 nm from day 1 to day 3. The continuation of the incubation does **not induce any further change** in the dichroic spectrum, and from day 3 to day 5, there is no longer any difference. A similar behaviour can be observed at acidic pH.

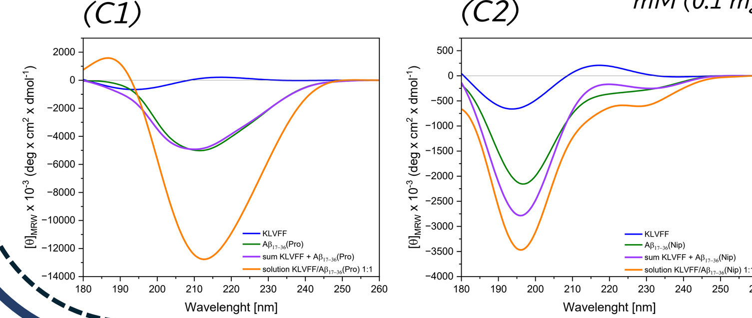




(up) 17-36pP (down) 17-36Nip *peptide dissolved in PB buffer 10 mM (0.5 mg/ml) **pH 2.1**, cell pathlength 1 mm, over **5 day**.*

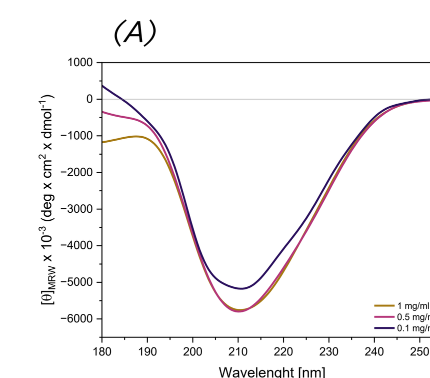


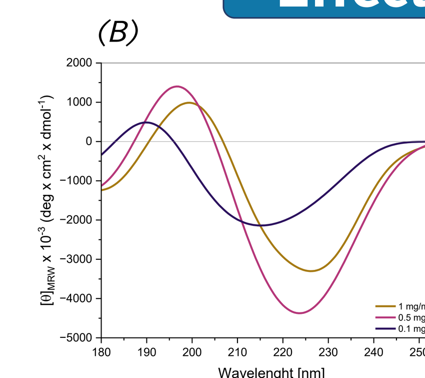
***Comparison** between calculated contribution and solution, for peptides (1) **17-36pP:KLVFF 1:1** (2) **17-36Nip:KLVFF 1:1** Peptides were dissolved in PB buffer 10 mM (0.1 mg/ml), pH: (A) 1.5 (B) 4.0 (C) 7.4, cell pathlength 1 mm.*



Further confirmation of the interaction of the KLVFF peptide with the two hairpins is obtained by comparing the measured spectrum for the hairpin/β-breaker mixture with the theoretical spectrum calculated by summing the contributions of the individual peptides at a given pH value.

Effect of concentration





17-36pP *peptide, dissolved in PB buffer 10 mM (1 mg/ml, 0.5 mg/ml, 0.1 mg/ml), pH (A) 7.4 (B) 2.1, cell pathlength 1 mm.*

17-36Nip *peptide, dissolved in PB buffer 10 mM (1 mg/ml, 0.5 mg/ml, 0.1 mg/ml), pH 2.1, cell pathlength 1 mm.*

The CD spectrum of both peptides in PB is not influenced by peptide concentration (as evidenced by the spectra of *17-36pP*).

Conversely, in **acidic conditions**, the CD spectrum is **markedly influenced** by peptide concentration, indicating the potential for peptide interactions.

Hairpin and β breaker

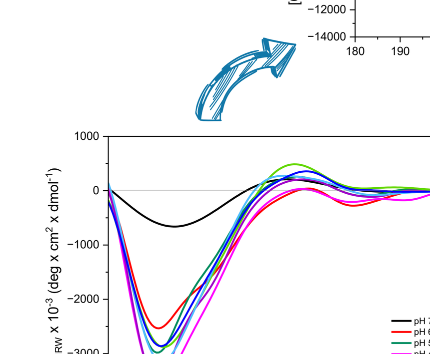
The capability of the **β-breaker KLVFF** peptide to interact with both ***17-36pP*** and ***17-36Nip*** peptides has been evaluated by CD spectroscopy at the different pH values (the studies were carried out in presence of 5% of trifluoroethanol -TFE- as a cosolvent because of the poor solubility of KLVFF in aqueous solution).

The influence of pH on the CD spectrum of KLVFF peptide was evaluated[6]. At pH 7.4, the CD spectrum of peptide shows a negative band at about 193 nm and a positive band at 215 nm, suggesting the presence of an unordered conformation. It has been reported that this conformation is always in equilibrium with other structures like **β-turns and -strands, and PP_{II} helix**, due to the closely proximity of the respective dihedral angle values[8].

The decrease in pH values is mainly accompanied by a **hyperchromic behaviour** of the molar ellipticity of the band from -500 to -3500 deg•cm²•dmol⁻¹.

17-36pP:KLVFF 1:1 solution titration *with H_2SO_4 0.05 M. Peptides were dissolved in PB buffer 10 mM (0.1 mg/ml), cell pathlength 1 mm.*

In the presence of the KLVFF peptide, the CD spectrum of the ***17-36pP*** peptide at neutral pH is characterized by the appearance of a positive band at about 195 nm and a red-shift displacement of the negative band from 210 to 213 nm, accompanied by an evident hyperchromic effect of the same. This effect persists throughout the range of pH values explored.



***KLVFF** peptide **titration** with H_2SO_4 0.05 M. Peptide was dissolved in PB buffer 10 mM (0.1 mg/ml), cell pathlength 1 mm.*

A qualitative representation of the effect of the presence of the KVLFF peptide can be obtained by reporting the ratio of the change in the dichroic signal over the total change, at a given wavelength, as a function of pH, for ***17-36pP*** (left) and ***17-36Nip*** (right).

17-36Nip:KLVFF 1:1 solution titration *with H_2SO_4 0.05 M. Peptides were dissolved in PB buffer 10 mM (0.1 mg/ml), cell pathlength 1 mm.*

Similarly, the presence of the peptide KLVFF also influences the CD spectrum of the peptide ***17-36Nip***, and in this case the effects can be observed as early as pH 6.2. For this peptide, too, the effect is essentially on the intensity of the dichroic bands, whereas at pH values below 4 units, the appearance of a weak positive band at 205 nm and an increase in the intensity of the negative band at around 230 nm are observed.

[1] Y. Yang, D. Arseni, W. Zhang, M. Huang; *SCIENCE*, **2022**, 375 (6577), 167-172
[2] S. Ruttenberg, A. Kreutzer, N. Truex, J. Nowick; *Biochemistry*, **2024**, 63, 212–218
[3] M. Teixidò, F. Albericio, E. Gitalt; *J. Peptide Res.*, **2005**, 65, 153–166
[4] Huck, J. Fisk, S. Gellman; *Org. Lett.*, **2000**, 17(2), 150-157
[5] B. Dasgupta et all.; *FEBS Letters*, **2007**, 581, 4529–4532
[6] L. Tjernberg, et all.; *J. of Biological Chemistry*, **1996**, *271* (15), 8545–8548
[7] . Woody, S. Venyaminov, N. Sreerama; *Protein Science*, **1999**, 8, 370-380

This work was supported by PRIN 2022 National Project (Grant N. 2022CAS9ZT, Development of HYbrid and Multifunctional Bio-conjugates against Alzheimer's Disease - HYMBAD)