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Lipophilicity of amyloid β-peptide 12–28 and 25–35 to unravel their ability to promote hydrophobic and electrostatic interactions

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ABSTRACT

The growing interest for peptide therapeutics calls for new strategies to determine the physico-chemical properties responsible for the interactions of peptides with the environment. This study reports about the lipophilicity of two fragments of the amyloid β-peptide, $A\beta_{25-35}$ and $A\beta_{12-28}$.

Firstly, computational studies showed the limits of log $D^{7.4}_{oct}$ in describing the lipophilicity of medium-sized peptides.

Chromatographic lipophilicity indexes (expressed as log *k'*, the logarithm of the retention factor) were then measured in three different systems to highlight the different skills of $A\beta_{25-35}$ and $A\beta_{12-28}$ in giving interactions with polar and apolar environments. CD studies were also performed to validate chromatographic experimental conditions.

Results show that $A\beta_{12-28}$ has a larger skill in promoting hydrophobic and electrostatic interactions than $A\beta_{25-35}$. This finding proposes a strategy to determine the lipophilicity of peptides for drug discovery purposes but also gives insights in unraveling the debate about the aminoacidic region of Aβ responsible for its neurotoxicity.

1. Introduction

In recent years, peptide-based drug discovery has gained a lot of relevance because of good safety, tolerability and efficacy of peptides. Consequently, there is an important focus on new approaches to improve the use of peptides in pharmaceutical research (Otvos and Wade, 2014; Fosgerau and Hoffmann, 2015).

Peptides behavior depends on their skill to interact with the environment (e.g. membranes and receptors) and on their aggregation properties. For instance, the amyloid β-peptide (Aβ, a peptide composed of 39–42 amino acids), is the most abundant component of β-amyloid plaques related to Alzheimer's disease (AD) (Hardy, 2009). Plaques formation is probably due to the skills of Aβ to form aggregates through the interaction with biomembranes (Wood et al., 2003; Meier and Seelig, 2008; Dies et al., 2014).

Lipophilicity studies provided a lot of information in the understanding of the interaction mechanisms between classical drugs (i.e. small organic compounds) and the environment (Testa et al., 1996) but poor information is reported in the literature about peptides.

We recently undertook a study to predict lipophilicity of small peptides (maximum length = 6 aminoacids) (Visconti et al., 2015). For these molecules, we found that they could be considered standard organic structures. However, the most relevant peptides in drug discovery are larger than six aminoacids and conformational effects are expected to strongly influence their behavior in the human body.

In this study, we characterize the lipophilicity of two flexible peptides of 11 and 17 aminoacids, respectively. In particular, we unravel the skills of two Aβ fragments, $A\beta_{25-35}$ and $A\beta_{12-28}$ in undertaking hydrophobic and polar interaction (the two main components of lipophilicity (El Tayar et al., 1992)) with polar and apolar environments. It should be recalled that shorter sequences of Aβ are often used as models of the full-length amyloid peptide, since they are easier to handle.

Computational studies were performed to highlight the limits of log $D^{7.4}_{oct}$ for characterizing the lipophilicity of the two medium-sized peptides.

Then we measured three chromatographic indexes (expressed as log *k'*) using one reversed-phase (RP) and two Hydrophilic Interaction Chromatography (HILIC) (Buszewski and Noga, 2012)

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systems. The idea is to use two distinguished sets of chromatographic systems to catch the different skills of the two peptides to engage hydrophobic (RP) and electrostatic interactions (HILIC) with different environments. The determination of lipophilicity indexes by chromatography is supported by a number of advantages (e.g. small amounts of material are required, impurities can be separated during the measurements, there is no need for concentration determination, the process is fast and can be easily automated) (Poole and Poole, 2003).

CD studies were undertaken to validate some experimental settings used in the chromatographic determinations.

2. Material and methods

2.1. Materials

$A\beta_{12-28}$ and $A\beta_{25-35}$ were purchased from Polypeptide Laboratories France (Strasbourg, France, www.polypeptide.com).

1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), acetonitrile (ACN), dimethylsulfoxide (DMSO) and ammonium acetate were purchased from Alfa Aesar GmbH & Co. (Karlsruhe, Germany, www.alfa.com).

Deionized water was used throughout.

2.2. Circular dichroism

Solutions of $A\beta_{12-28}$ and $A\beta_{25-35}$ in the concentration range 30–400 μM, both in pure HFIP and 10 mM PBS buffer at pH 7.4 + 10% HFIP, were scanned in the far-UV spectral range (four accumulations) over the wavelength region 180–260 nm with a scanning speed of 50 nm/min using a Jasco J-815 spectropolarimeter equipped with a Xe arc lamp. Spectra were recorded in a quartz circular cuvette (optical path length 0.1 cm). Buffers baselines were subtracted for each measurement.

Secondary structure was estimated from the mean residue ellipticity [θ] with the CDNN CD spectra deconvolution software (Version 2.1, Copyright (C) 1997 Gerald Böhm).

2.3. Chromatography

The mobile phase consisted of 20 mM ammonium acetate buffer at pH 7.0 and acetonitrile in varying proportions. For all mobile phases, the given pH is the pH of the buffer before the addition of organic modifier.

The flow rate was 1 mL/min. The solvent front were used to determine t_0, i.e., the dead time in RP systems, toluene was used to determine t_0 under HILIC conditions.

HFIP solutions of both peptides were prepared (concentration range of 50–100 μg/mL) and injected in the HPLC systems. The choice of HFIP was made on the basis of preliminary tests, which evidenced the modest solubility of the two peptides in phosphate buffered saline (PBS) and DMSO. Conversely, they were largely soluble in HFIP.

The retention time (t_R) were measured on three columns: (1) PLRP-S polymeric reversed phase column (Agilent, 5cm × 4.6 mm, 5 μm packing, 100 Å pore size); (2) ZIC-HILIC column (sulfoalkylbetaine zwitterionic phase on a silica gel support, 10 cm × 4.6 mm, 5 μm packing, 200 Å pore size) from SeQuant (Umeå, Sweden) and (3) ZIC-cHILIC column (phosphorylcholine zwitterionic phase on a silica gel support, 10 cm × 4.6 mm, 3 μm packing, 100 Å pore size) from SeQuant (Umeå, Sweden). Measures were performed in triplicate.

The chromatographic indexes are expressed as log k' (Eq. (1))

$$\log k' = \log\left(\frac{t_R - t_0}{t_0}\right) \quad (1)$$

where k' is the retention factor, t_R is the retention time and t_0 is the dead time.

A HPLC Varian ProStar instrument equipped with a 410 autosampler, a PDA 335 LC Detector and Galaxie Chromatography Data System Version 1.9.302.952 was used.

2.4. Ionization and calculated lipophilicity

Ionization constants were calculated with MoKa (Version 2.5.4, http://www.moldiscovery.com); log $D^{7.4}_{oct}$ values were calculated with a model recently published by some of us (Visconti et al., 2015).

2.5. Molecular dynamics simulations

All simulations and analysis described below were done using the AMBER14 package that also includes the trajectory analysis software AmberTools and the module xLEaP used to prepare starting structures (Case et al., 2012). In particular, MM minimizations and MD simulations were performed using sander and pmemd modules, respectively.

The starting structures of $A\beta_{12-28}$ and $A\beta_{25-35}$ were obtained from the crystallographic structure of $A\beta_{1-42}$ (PDB id: 1IYT) after deleting unnecessary aminoacids. MD simulations were performed with constantprotonation states for titrable residues. Peptides were modeled in the electrical state dominating at pH 7.0. Histidine was considered neutral and the ε-tautomer was used in the simulations according to default AMBER choice and to MoKa prediction.

Input files were prepared submitting all starting structures to the xLEaP module. The ff99SB force field was employed.

During the chromatographic experiments, peptides experience different environments that depend on the eluent composition. To obtain reliable simulations we tried to approach the experimental conditions used to register chromatograms. In particular, we considered two limit conditions. In the first, epsilon was fixed at 78.5 to mimic an aqueous environment. The second epsilon was set at 37.5 to mimic acetonitrile. Solvation effects for the investigated solvents (water and acetonitrile) were incorporated using the pairwise Generalized Born model with parameters described by Tsui and Case (Tsui and Case, 2000). This model uses the default radii set up by xLEaP.

Before launching MD simulations, all atoms were optimized without any constrain (500 cycles of steepest descent followed by 500 cycles of conjugate gradient minimization). After minimization, all systems were gradually heated from 0 to 325 K with a time step of 0.5 fs over a period of 35 ps. The temperature plot was used to confirm the attainment of the equilibrium of the heating phase. Finally, 50 ns MD simulations were performed with a time step of 2 fs. During the MD simulations, the atom coordinates were saved every 500 steps. All the covalent bonds involving hydrogen atoms were constrained with the SHAKE algorithm and the Berendsen thermostat was used, both as implemented in AMBER14. For temperature control a heat bath coupling of 1.0 ps and 0.5 ps were used during heating and MD simulation, respectively.

The MD Movie tool of USCF Chimera (Version 1.10, http://www.cgl.ucsf.edu/chimera) was used to cluster the trajectories based on pairwaise best-fit root-mean-square deviations (RMSDs) calculated on the backbone atoms and to identify a representative frame for each cluster. For any peptide we considered those clusters that taken together include about 80% of the entire population of conformers.

For validation purposes it should be mentioned that the most stable structure of $A\beta_{1-42}$ obtained with our approach (data not shown) is in agreement with that reported in the literature