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A left-handed 3$_1$ helical conformation in the Alzheimer Aβ(12–28) peptide

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Abstract **We show for the first time that the secondary structure of the Alzheimer β-peptide is in a temperature-dependent equilibrium between an extended left-handed 3$_1$ helix and a flexible random coil conformation. Circular dichroism spectra, recorded at 0.03 mM peptide concentration, show that the equilibrium is shifted towards increasing left-handed 3$_1$ helix structure towards lower temperatures. High resolution nuclear magnetic resonance (NMR) spectroscopy has been used to study the Alzheimer peptide fragment Aβ(12–28) in aqueous solution at 0°C and higher temperatures. NMR translation diffusion measurements show that the observed peptide is in monomeric form. The chemical shift dispersion of the amide protons increases towards lower temperatures, in agreement with the increased population of a well-ordered secondary structure. The solvent exchange rates of the amide protons at 0°C and pH 4.5 vary within at least two orders of magnitude. The lowest exchange rates (0.03–0.04 min^{-1}) imply that the corresponding amide protons may be involved in hydrogen bonding with neighboring side chains.**

Key words: Alzheimer β-peptide fragment 12–28; Left-handed 3$_1$ helix; PII helix; Circular dichroism; Nuclear magnetic resonance

1. Introduction

Amyloid diseases are associated with accumulation of extracellular amyloid deposits in the brain. For Alzheimer's disease the major component of the plaques and intermediate toxic species is the Aβ peptide, 39–43 residues long, formed by enzymatic cleavage from the much larger amyloid precursor protein [1–3]. Aβ has a high propensity for formation of aggregates with a high content of β-sheet secondary structure. Eventually regular fibrillar structures are formed, which are rich in β-sheet structure. Despite large efforts involving e.g. fiber X-ray diffraction [4], solid-state nuclear magnetic resonance (NMR) [5] and solution NMR [6,7], the structural understanding of the processes involved in aggregation and fiber formation is limited [8]. In previous studies we and others [9–11] have studied fragments of the full-length peptide, and particularly concentrated on fragments containing the central hydrophobic cluster (16–21), which appears to be essential for aggregation. The Aβ(12–28) peptide fragment has the sequence VHHQ KLVFF AEDVG SNK. The previous studies using circular dichroism (CD) and high resolution NMR [6,9–11] have suggested that such fragments, like the full-length peptide, at low temperatures are largely unstructured monomers, which aggregate and form β-sheets at higher temperatures. Here we have studied Aβ(12–28) at very low peptide concentration and low temperature. The results show that monomeric Aβ(12–28) in aqueous solution at 0°C is in a reversible equilibrium between a conformation which has the characteristics of an ordered left-handed 3$_1$ helix (like in polyproline type II, denoted PII helix) and a flexible random coil conformation, which becomes more predominant at higher temperatures.

2. Materials and methods

2.1. Materials

The Alzheimer β-peptide fragment Aβ(12–28) was purchased from Neosystem (Strasbourg, France). The purity of the peptide was tested by high performance liquid chromatography, mass spectrometry and NMR. α-Cyclodextrin was purchased from Sigma-Aldrich. The quantities of solid materials for all samples were determined by weight.

2.2. NMR spectroscopy

The samples for NMR experiments were prepared at a concentration of 1.0 mM of Aβ(12–28) by directly dissolving solid material at 5°C in H_2O or D_2O buffer (10 mM sodium acetate-d_3, pH 4.5). The pH was measured with a combination glass microelectrode, designed for NMR (Orion model 9826BN, with model 320 pH-meter). Unless otherwise stated, all the pH values are *uncorrected* meter readings. For samples dissolved in D_2O, a correction of +0.4 units should be added to the readings in order to get the corresponding pD value. Under these conditions, the NMR spectra were resolved and the samples were stable over a period of several weeks, provided that the storage was at a low temperature. NMR measurements were performed using 5 mm tubes and standard pulse sequences on Varian Innova 600 and 800 MHz spectrometers.

Translational diffusion coefficients were measured at 1°C and 25°C using the 600 MHz Varian Innova spectrometer with a *z*-axis gradient coil. For all experiments 20 values of gradient strength were used, from 0.005 to 0.3 T/m. All spectra were baseline corrected. The pulsed field gradients were calibrated using a standard sample, 1% H_2O in D_2O and 1 mg/ml $GdCl_3$. The HDO diffusion coefficient in D_2O at 25°C has a value of 1.90×10^{-9} m^2/s, which was used for calibration of the gradients [12].

2.3. CD spectroscopy

A Jasco J-720 spectropolarimeter and a PTC-343 temperature controller were employed. The spectral range was 190–250 nm with a resolution of 0.2 nm and a bandwidth of 1 nm. A scan speed of 100 nm/min with 2 s response time was employed. A quartz cell with 1 mm optical path was used. The spectral contribution from the background was subtracted and the results were expressed as mean residue molar ellipticity [θ].

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3. Results and discussion

3.1. CD

Fig. 1 shows CD spectra of a dilute aqueous solution of 30 μM Aβ(12–28) recorded at various temperatures from 0°C to 60°. At this low concentration and low ionic strength the sample is stable and does not precipitate. The temperature-dependent changes in the CD spectra shown in Fig. 1 are completely reversible. There is an isodichroic point at 208 nm, typical of a two-state equilibrium. Comparing the present series of CD spectra with literature data on left-handed 3_1 helices [13–17], the results indicate that the equilibrium involves a random coil structure at high temperatures with increasing contributions of a left-handed 3_1 helix at low temperatures. The left-handed 3_1 helix is an extended helix with minor but significant CD spectral features that distinguish it from a random coil structure: a weak positive band at 220 nm and a deep negative band centered around 196–198 nm distinguish the left-handed 3_1 helix spectrum from that of a random coil spectrum, which has a negative shoulder around 220 nm and a weaker negative band centered around 195 nm [13–17].

Quantitation of the amount of left-handed 3_1 helix has in previous studies been based on observed mean residual ellipticity values, of either the negative 196–198 nm band or the positive band around 220 nm. In both cases considerable uncertainties arise because of calibration problems. Here we have used both wavelength regions. At 196 nm the mean residue ellipticity $[\theta]_{196}$ is −36 300 deg cm^2/dmol for a fully ordered left-handed 3_1 helix [18] and −7600 deg cm^2/dmol for a random coil [14]. The CD spectrum of Aβ(12–28) at 0°C has $[\theta]_{196}$ −11 800 deg cm^2/dmol, indicating about 15% left-handed 3_1 helix. Using instead the long wavelength band and its maximum $[\theta]_{222}$ of about 500 deg cm^2/dmol inserted in the equations given in [19] or [20], we calculated the population left-handed 3_1 helix to be either 40% or 48%. An average estimate (with a large error margin) would be about 30% left-handed 3_1 helix in Aβ(12–28) under the present conditions (0°C, pH 4.5).

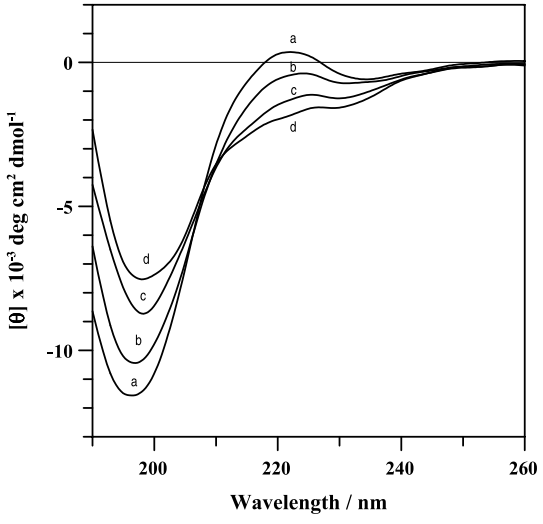

Fig. 1. Far UV CD spectra of 30 μM Aβ(12–28) in 10 mM acetate buffer at pH 4.5. The peptide was dissolved at 5°C and spectra (a–d) were recorded at 0°C, 20°C, 40°C and 60°C. The CD spectra were recorded with 1 mm optical path length and were baseline corrected using a buffer sample.

In a separate experiment we also studied the short peptide KKLVFFA, derived from the hydrophobic cluster of Aβ. In this case the temperature variation of the CD spectrum was even more clearcut, showing the left-handed 3_1 helix–random coil equilibrium (data not shown).

3.2. NMR

We used high resolution NMR to further study the structural properties of the Aβ(12–28) peptide. Our earlier NMR studies [11] on 0.9 mM Aβ(12–28) at 5°C and 25°C in D_2O had shown that the sample had a high molecular weight NMR-invisible fraction (30–40%), while the remainder is observable by NMR and was shown to be monomeric by diffusion studies at both temperatures. Fig. 2 shows the amide proton region of a one-dimensional ^{1}H NMR spectrum of 1 mM Aβ(12–28) dissolved in H_2O (A) at 25°C and (B) at 0°C, and (C) in D_2O at 0°C directly (8 min) after dissolving the sample. The assignments (Fig. 2) were obtained from two-dimensional ^{1}H nuclear Overhauser effect spectroscopy and total correlation spectroscopy (TOCSY) spectra in H_2O solvent using standard procedures [21]. Partial TOCSY spectra are shown in Fig. 3. Large upfield shifts (up to 0.2 ppm) of the amide proton resonances were observed for the H_2O sample when the temperature was increased from 0°C to 25°C. We also observed large variations in the amide proton exchange times for the peptide freshly dissolved in D_2O (Fig. 2C). The measured exchange times of the slowly exchanging amide protons are indicated in Fig. 2C. The longest exchange times (25 min for V18 and 38 min for K28) correspond to exchange rates of about 0.03–0.04 min^{-1}.

The chemical shift dispersion of the amide proton resonances is almost the same at 0°C and at 25°C (Fig. 2A,B). However, the CD studies suggest that this structure is partially a left-handed 3_1 helix. Compared with literature data on the expected chemical shift changes for amide protons from a random structure to a left-handed 3_1 helix [14], the changes observed here (ca. 0.2 ppm downfield at 0°C compared to 25°C) are in the same direction but generally larger. This may be explained by the lower temperature (0°C) used here than in the earlier study where the NMR parameters of a left-handed 3_1 helix were characterized (10°C) [14].

To ensure that the peptide observed here by NMR is in a monomeric state, translation diffusion measurements were performed with ^{1}H pulsed field gradient NMR using calibrated magnetic field gradients. High accuracy in the measured diffusion coefficients was achieved by fitting a gradient strength distribution function to the reference data in the calibration experiments [12]. At 25°C the diffusion coefficient for Aβ(12–28) was found to be 2.18×10^{-10} m^2/s, in good agreement with earlier measured monomeric diffusion of the same peptide [11,22]. The diffusion coefficient at 1°C is 1.09×10^{-10} m^2/s. To account for temperature and viscosity changes at the lower temperature, the diffusion coefficients of water and α-cyclodextrin were measured at 25°C and 1°C, as references. The ratio (2.07) between the respective diffusion coefficients at 25°C and 1°C reflects the changes in temperature and viscosity, for substances with a constant hydrodynamic radius, and any temperature-dependent change in hydrodynamic radius of the peptide could be estimated. We found that Aβ(12–28) has a slightly smaller hydrodynamic

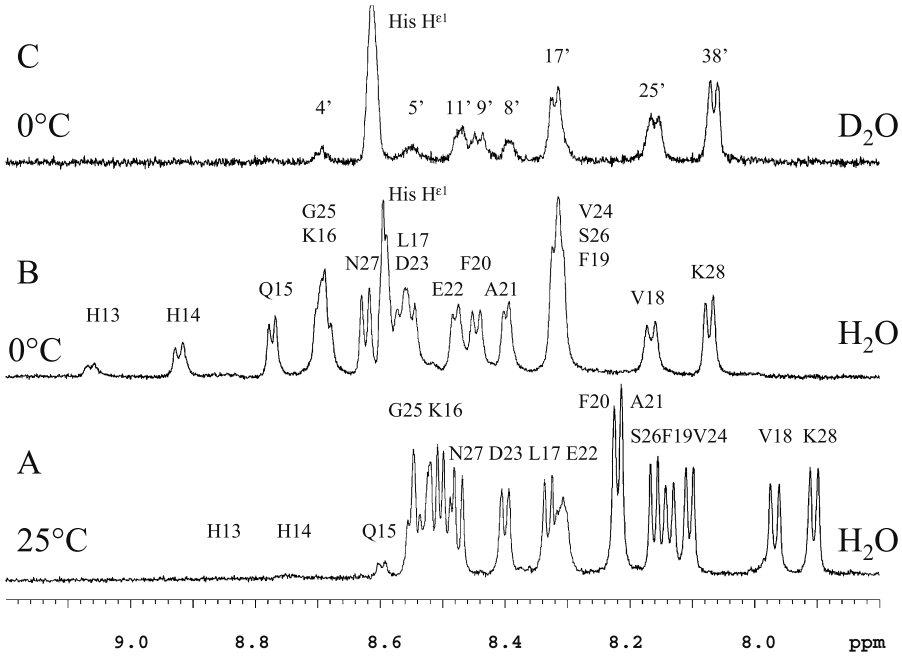

Fig. 2. Amide proton region of 600 MHz ^{1}H NMR spectra of 1 mM Aβ(12–28) in 10 mM acetate-d_3 buffer, pH 4.5, (A) at 25°C in H_2O, (B) at 0°C in H_2O and (C) 8 min after dissolving in D_2O. Numbers indicate exchange lifetimes in minutes. Residues are labeled using single letter notation. Amide protons in residues H13 and H14 are broadened due to exchange with water and suppressed due to water presaturation at 25°C.

radius at 1°C than at 25°C, $r_H(1°C) = 0.96 r_H(25°C)$. The diffusion coefficients show that the peptide is in a monomeric state at both temperatures.

Under the present conditions of temperature and pH, the solvent exchange rates of the amide protons of an amino acid without ordered hydrogen bonds (e.g. in a dipeptide) are expected [23] to be on the order of 0.1–1 min^{-1}. These rates are in general agreement with the rates observed here (Fig. 2C), but the large variation of rates and the observation of certain rates < 0.1 min^{-1} suggest that the most slowly exchanging amide groups in Aβ(12–28) may be particularly protected. The slow exchange could possibly be related to formation of hydrogen bonds to neighboring side chains (e.g. for K28, cf. [9]). Generally a left-handed 3_1 helix should be devoid of intramolecular main chain hydrogen bonds [14].

Our earlier NMR work [11] on Aβ(12–28) in D_2O showed a non-explained sequence periodic variation of the H^α resonances, as well as reversible, small, but significant temperature-dependent chemical shift variations of the H^α resonances involving residues 18–23. These chemical shift properties of monomeric Aβ(12–28) can now be interpreted in terms of a partial left-handed 3_1 helix shifting towards more complete

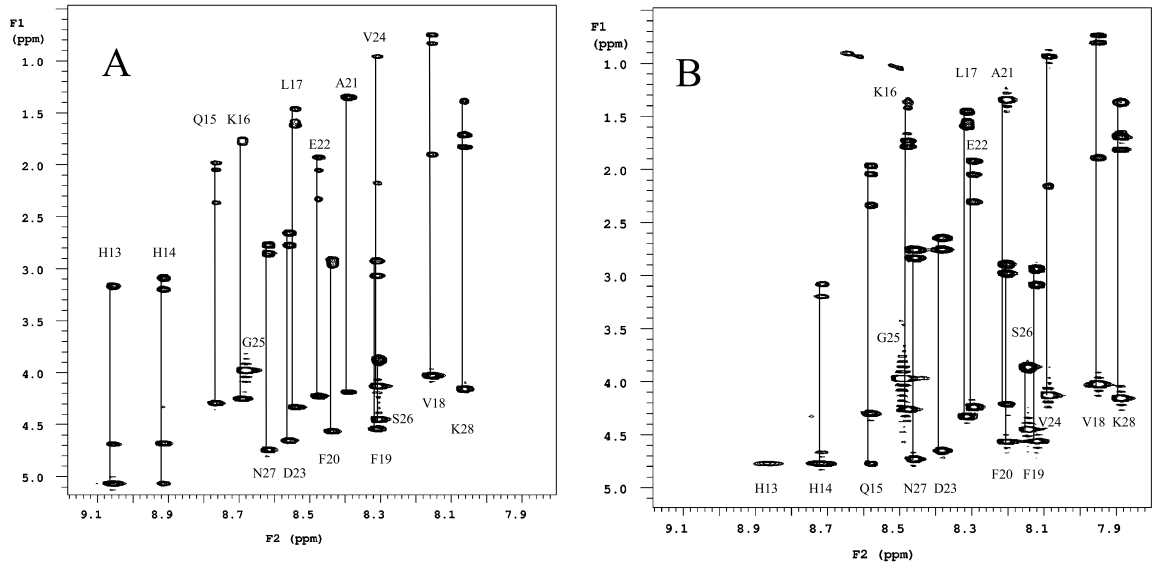

Fig. 3. Partial 800 MHz ^{1}H NMR TOCSY spectra of 1 mM Aβ(12–28) in 10 mM acetate-d_3 buffer, in H_2O, pH 4.5, (A) at 0°C, (B) at 25°C. Amide protons in residues H13, H14 and Q15 show cross-peaks to water.

random coil and then further to β-strand structure towards higher temperatures. Literature data [14] show that a left-handed 3_1 helix at 10°C should be characterized by a 0.01–0.03 ppm upfield shift of the H^{α} resonances from the random coil values.

Available data suggest that the observations for the Aβ(12–28) fragment should also hold for the full-length peptide [9]. The temperature-dependent CD spectra reported for Aβ(1–40) [24] show similar features as those presented here. NMR structural work [6] in aqueous solution at 8°C on the whole Aβ(1–40) peptide has shown a weakly defined, but non-random structure for the 16–24 segment (as well as for other segments outside the present study), which may be related to the results shown here.

4. Conclusions

Our results show the partial presence of an ordered secondary structure in the Aβ(12–28) peptide at low temperatures: an extended left-handed 3_1 helix interconverts with a flexible random coil conformation. Conditions such as low temperature which favor the left-handed 3_1 helix also increase the sample stability with a low propensity of the peptide to undergo the transition to β-structure. Overall, the emerging picture is that many peptides are in fact partial left-handed 3_1 helices at low temperatures, and one has to be aware that a putative random coil peptide is not always random (cf. comment in [16]). One obvious consequence is that certain reference spectra and parameters characteristic for a random coil state in CD and NMR libraries may have to be reexamined.

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