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COMPARATIVE ANALYSIS OF HUMAN AND DUTCH-TYPE ALZHEIMER β-AMYLOID PEPTIDES BY INFRARED SPECTROSCOPY AND CIRCULAR DICHROISM

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The 42 amino acid βA4 peptide is the major constituent of the senile plaques, one of the hallmark neuropathological lesions of Alzheimer's disease. While C-terminally truncated variants were shown to be present in normal body fluids, a single Glu → Gln change in the 39 amino acid form of βA4 results in accelerated fibril formation in the brains of patients with Dutch-type hereditary cerebral hemorrhage with amyloidosis. In this study we used Fourier-transform infrared and circular dichroism spectroscopies on synthetic peptides to demonstrate that this mutation results in altered secondary structure in membrane mimicking solvents, characterized by a considerably higher β-structure content for the mutant peptide. Moreover, extreme high and low pH were less effective in eliminating the β-conformation for the Dutch-variant than for the normal human sequence.

The brains of AD patients are characterized by abundant fibrous lesions, i.e., SPs, NFTs, and neuropil threads (1-3). Although not restricted to AD, the burden of SPs and NFTs correlates well with the dementia (4, 5). SPs are extracellular amyloid deposits consisting mostly of a 42 amino acid polypeptide called βA4 (6), which is derived from one or more larger transmembrane proteins, i.e., the β-APP (7). Similar deposits have been detected in aged monkeys, dogs, and polar bears, but rarely have they been found in rats and mice (8). Three amino acid substitutions are found in the βA4 region of rodents. These amino acid differences may account for the inability of the rodent peptide to form amyloid fibrils *in situ* (9). A 39 amino acid residue βA4 with another point mutation is detected in patients with Dutch-

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The abbreviations used are: AD, Alzheimer's disease; SPs, senile plaques; NFTs, neurofibrillary tangles; HCHWA-D, Dutch-type hereditary cerebral hemorrhage with amyloidosis; APP, β-amyloid precursor protein; CD, circular dichroism; FT-IR, Fourier-transform infrared; TFE, 2,2,2-trifluoroethanol; OcGlc, octyl β-D-glucopyranoside; TFA, trifluoroacetic acid.

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type HCHWA-D, for which an accelerated fibril formation is observed. The amino acid sequences of the three peptides are

		1 5 10 13 22 39 42
Human:	βA4-H:	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Rodent:	βA4-R:	D G F R A
Dutch:	βA4-D:	D Q V(A).

Normal processing of APP leads to non-amyloidogenic fragments (10,11) by a cleavage mechanism that appears to be more dependent on the conformation in the membrane than specific to APP (12). As a result, minor amino acid changes in the βA4 region may affect the appropriate enzyme activity.

Recent data from several laboratories indicate that a 40 amino acid N-terminal fragment of βA4 can be recovered from serum and cerebrospinal fluid of both control and AD patients, and is secreted by a variety of transfected and non-transfected cells (13-15). Moreover, a comparison of the solution conformations of βA4-H (1-39) and βA4-H (1-42) suggests that the last three C-terminal amino acids are crucial to amyloid deposition (16). Based on this, one would not expect the preferential deposition of βA4-D. Nevertheless, the existence of these deposits in HCHWA-D underlines the importance of the $Glu^{22} \rightarrow$ Gln change in the conformation of the amyloidogenic peptide. In this study, we compare the conformation of the βA4-H and βA4-D peptides in membrane-mimicking solvents as detected by CD and FT-IR. Since the secondary structure of βA4-H exhibits a strong chain-length dependence (16), and the true differences prevail only in peptides of equal size, we chose to study an extended, 42 amino acid-long analog of βA4-D. A recent study, using electron microscopy, FT-IR, and fiber X-ray diffraction (17), scrutinized the N-terminal 40 amino acid fragments of the human and rodent βA4 peptides, and a 40 amino acid analog of βA4-D. In spite of the thorough pH-dependence studies the authors did not find major differences in the secondary structure of the human and rodent sequences, which may be related to the lack of the crucial two C-terminal amino acids. Moreover, we use solvent systems that mimic the natural membrane-like environment (instead of pure water only) to obtain insights into the accelerated fibril formation of the Dutch variant.

MATERIALS AND METHODS

Peptide Synthesis, Purification, and Characterization - All details of the preparation of the βA4 peptides were recently published (9). Briefly, the peptides were assembled on a Milligen 9050 automated synthesizer using standard Fmoc-protocol. After cleavage, the peptides were dialyzed in a 3,500 Da cutoff tubing followed by a reversed-phase HPLC purification using a C18 column and a linear gradient of acetonitrile in 0.1% aqueous TFA. The integrity of the peptides was verified by amino acid analysis and fast atom bombardment mass spectroscopy.

CD Measurements - CD spectra were taken on a Jasco J-720 circular dichrograph at room temperature in a 0.2 mm path-length cell. Double distilled

water, spectroscopy grade TFE, and acetonitrile were used as solvents. Peptide concentration was 0.5 mg/ml. Mean residue ellipticity ($[\Theta]_{MR}$) is expressed in deg cm^2/dmol by using a mean residue mass of 110 Da. The spectra in Fig. 4 are baseline corrected and smoothed by using the algorithm provided by Jasco.

FT-IR Spectroscopy - Infrared spectra were recorded on a Digilab FTS-60 instrument at room temperature. Measurements were performed in solutions of spectrograde D_2O, TFE, acetonitrile or OcGlc, and mixtures thereof. All data were obtained from freshly prepared solutions at peptide concentrations between 2 and 3 mg/ml, and spectra were recorded 15-20 min after peptide dissolution. The peptide solutions were placed in a demountable cell with CaF_2 windows and a path length of 45 μm. For each sample, 512 inteferograms were co-added and Fourier-transformed to give a resolution of 2 cm^{-1}. Infrared spectra of the solvents were obtained under the same conditions, and were subtracted from the spectra of the peptides in the respective solvents. The infrared spectra in Figs. 1-3 are shown after band-narrowing by Fourier self-deconvolution by use of identical deconvolution parameters for all spectra (half-bandwidths of 16 cm^{-1} and band-narrowing factor of k=1.75). Band narrowing leads to a better visualization of overlapping bands, without affecting their integrated intensities (18). All peptides contained small and comparable amounts of the counterion TFA which gives rise to an infrared band at 1673 cm^{-1}. TFA was not subtracted, but used as an intensity standard for comparing the spectra.

RESULTS AND DISCUSSION

Infrared Spectroscopic Studies - Both the human and Dutch-type βA4 peptides were insoluble in D_2O and formed aqueous suspensions over the pH range of 2-10. The infrared spectra of both peptides in this pH range are dominated by strong infrared bands at 1623 and 1673 cm^{-1}, a weak band at 1696 cm^{-1}, and a shoulder near 1652 cm^{-1}. As an example, Fig. 1a shows the infrared spectra of the Dutch-type (full line) and the human peptide (broken line) measured as suspension in D_2O at pH 7. The strong infrared band near 1673 cm^{-1} seen in Fig. 1 (and in all other spectra) arises from small amounts of TFA in the peptide solutions. The other bands in the region between 1600 and 1700 cm^{-1} can be assigned to amide I band components (essentially C=O stretching vibrations of the amide functional group). The strong and intense band at 1623 cm^{-1} and the weak band at 1696 cm^{-1} are characteristic of antiparallel β-sheet structures (19). These bands show that both peptides adopt primarily β-structures. The weak bands near 1652 cm^{-1} arise from α-helical and unordered conformations (19).

Fig. 1b shows the infrared spectra of the human and Dutch-type βA4 peptides in D_2O at pH 12. Under these experimental conditions, only the Dutch-type peptide possesses a significant amount of antiparallel β-structure as indicated by the infrared band at 1629 cm^{-1} (see full line in Fig. 1b). The major band in the spectrum of human βA4 peptide is centered at 1642 cm^{-1} and is characteristic of amide I bands in unordered structures. A small amount of α-helical structure is indicated by the shoulder at 1652 cm^{-1}.

We have also carried out a conformational analysis of common human versus Dutch-type βA4 peptides in various membrane-mimicking solvents. In

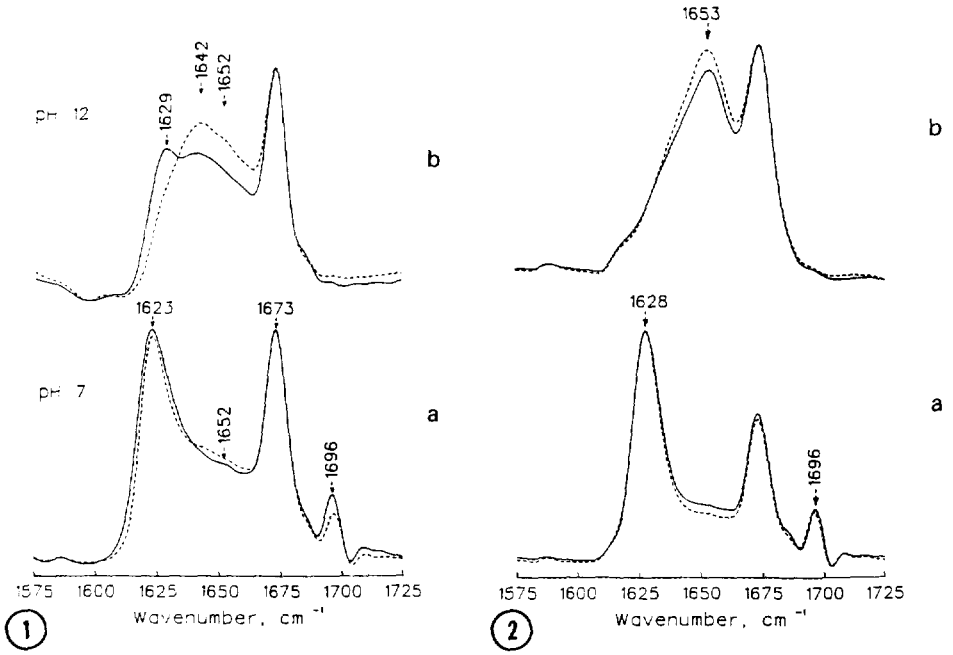

Figure 1. FT-IR spectra of the human βA4 (broken line) and the Dutch-variant (solid line) in aqueous conditions at different pH. Panel **a**: at pH 7, panel **b**: at pH 12.

Figure 2. FT-IR spectra of the human (broken line) and Dutch-type (solid line) βA4 peptides in membrane-mimicking solvents at neutral pH. Panel **a**: in 10% OcGlc, panel **b**: in aqueous (50%) TFE.

OcGlc micelles, both peptides formed clear solutions and exhibit spectra characteristic of nearly 100% extended β-strands (Fig. 2a), as indicated by the very strong band at 1628 cm^{-1} and the weaker band at 1696 cm^{-1}. The proportion of α-helical and/or unordered conformations is very small as indicated by the negligible intensity between 1645 and 1655 cm^{-1}. The infrared spectra of both βA4-peptides in OcGlc micellar solutions represent the highest amount of extended antiparallel β-strands we have observed for these peptides, much higher than in aqueous suspension (Fig. 1a).

Quite opposite results were obtained in TFE, a solvent frequently used in CD and NMR studies because it induces the formation of α-helical conformations in peptides and proteins. In aqueous TFE (1:1; v/v) both βA4 peptides were true solutions and exhibit spectra characteristic of predominantly α-helical structures as indicated by the strong band centered at 1653 cm^{-1} (Fig. 2b). The lack of an infrared band in the range of 1625-1630 cm^{-1} excludes the presence of a significant amount of β-structure.

The third solvent for simulating an interface-like situation was acetonitrile, a non-hydrogen bonding organic solvent. In acetonitrile/water mixtures, both peptides where true solutions, whereas the peptides were not soluble in water or

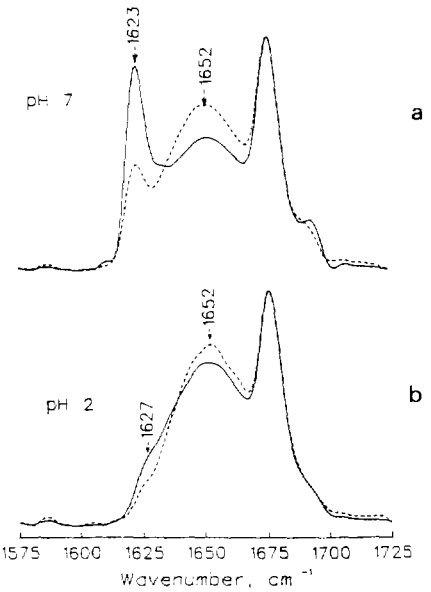

Figure 3. FT-IR spectra of the human βA4 (broken line) and the Dutch-variant (solid line) in aqueous (50%) acetonitrile at different pH. Panel **a**: at pH 7, panel **b**: at pH 2.

acetonitrile alone. Fig. 3a shows infrared spectra of human and Dutch-type βA4 peptides in a 1:1 (v/v) mixture of D_2O and acetonitrile at neutral pH. The characteristic bands at 1623 and 1696 cm^{-1} are more pronounced in the spectrum of βA4-D (full line in Fig. 3a). Therefore, from a comparison of the two spectra of βA4-D and βA4-H it is evident that under these experimental conditions the Dutch-type peptide possesses a significantly higher proportion of antiparallel β-structure than the common human peptide. The broad band centered at 1652 cm^{-1} is assigned primarily to α-helical structures, although some of the band intensity likely originates from unordered structures. The amount of β-structure in both peptides is reduced at pH 2 (Fig. 3b), but still slightly higher for the Dutch-type (full line in Fig. 4). This indicates that in the membrane-mimicking acetonitrile/water mixture the tendency for the formation of β-sheet structures is higher in peptide βA4-D than in peptide βA4-H.

CD Spectroscopic Studies - Since the peptides were not soluble in pure water, CD spectra were taken in water/organic solvent mixtures. In a 1:1 (v/v) mixture of water and TFE at neutral pH, both peptides exhibit CD spectra characteristic for a dominant α-helical structure as indicated by the pronounced negative bands at 222 and 208 nm and a positive band at 192 nm (Fig. 4a). A negative 222 nm band, and a negative and a positive band at 208 nm and 192 nm, respectively, are typical for CD spectra of α-helices (20). A content of 31% α-helix for the Dutch-type peptide and of 48% α-helix for the human peptide is estimated from the intensity of the 208 nm

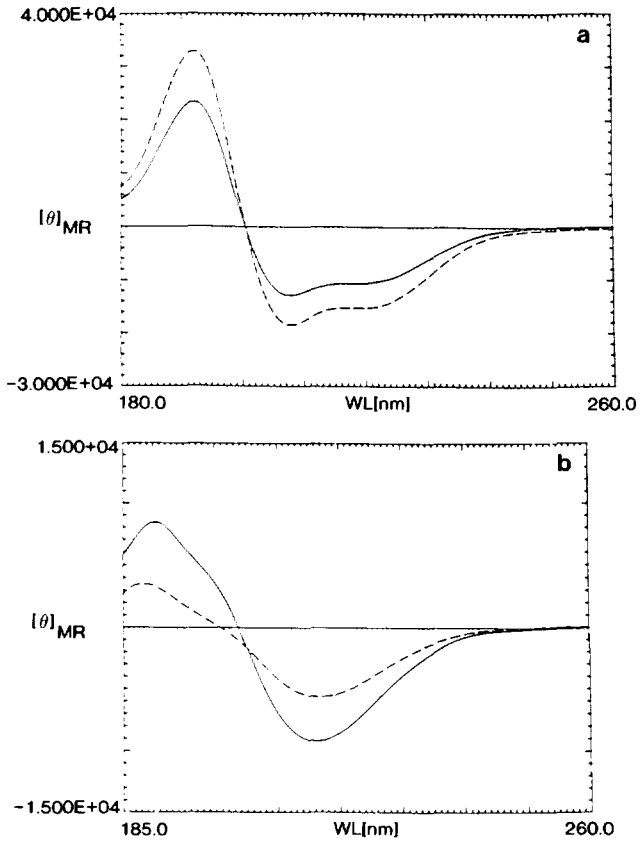

Figure 4. CD spectra of the human (broken line) and Dutch-type βA4 peptides in membrane-mimicking solvents at neutral pH. Panel **a**: in aqueous (50%) TFE, panel **b**: aqueous (50%) acetonitrile.

band according to Greenfield and Fasman (21). These quantitative differences should not be overinterpreted, because the calculation is based on the weight of the lyophilized peptide powders. The highly hydrophobic nature of the βA4 peptides excludes the possibility of accurate determination of peptide concentration by amino acid analysis or HPLC (9).

Both βA4 peptides exhibit more β-pleated sheet-type CD spectra in acetonitrile/water mixtures (Fig. 4b). The CD spectra of polypeptides in β-pleated sheets feature a single negative band near 216 nm and a positive band around 195 nm (22). Pronounced negative and positive bands at 216 and 190 nm, respectively, are present in the CD spectrum of the Dutch-type peptide. In comparison to the Dutch-type, a 40% decrease in intensity of the negative band at 216 nm is observed for the human peptide, suggesting a significantly smaller amount of β-structure for the human sequence.

In summary, here we have presented spectroscopic evidences for a preferred tendency of the βA4 peptide originated from HCHWA-D to aggregate into β-

structures, compared to the common human βA4 peptide. It also appears that the elimination of the β-structure in βA4-D is even more difficult than in other βA4 sequences.

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