Reversible Random Coil–β-Sheet Transition of the Alzheimer β-Amyloid Fragment (25–35)†

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ABSTRACT: The β-amyloid protein (39–43 amino acid residues) is the major constituent of the amyloid deposits found in brain of patients with Alzheimer's disease. Using circular dichroism spectroscopy, we have studied the secondary structure and the aggregation of fragment 25–35 of the β-amyloid protein (βAP(25–35)OH) under a variety of conditions. βAP(25–35)OH in solution at pH 4.0 or 5.5 exhibits a concentration-dependent random coil ⇄ β-sheet transition. The equilibrium is characterized spectroscopically by an isodichroic point and can be described quantitatively by a simple association model with association constants between 1.8×10^4 M^{-1} (non-cooperative model, nucleation parameter $\sigma = 1$) and 2.9×10^4 M^{-1} (cooperative model, $\sigma = 0.2$). The enthalpy of association is $\Delta H \approx -3$ kcal/mol as determined by titration calorimetry. The equilibrium is shifted completely toward β-structured fibrils at pH 7.4 where the Met-35 carboxyl group is fully charged. In contrast, removal of the charged carboxy terminus by amidation locks the equilibrium in the random coil conformation. Model calculations suggest an antiparallel β-sheet structure involving residues 28–35 which is stabilized at both ends of the β-sheet by ion pairs formed between Lys-28 and Met-35. Removal of fibrils via millipore filtration leads to solutions with random coil monomers only. Seeding these solutions with a few fibrils establishes a new random coil ⇄ β-sheet equilibrium.

The β-amyloid protein (βAP) constitutes the major component of senile plaques and cerebrovascular amyloid deposits found in the brain of patients with Alzheimer's disease (Glenner et al., 1984; Masters et al., 1985a,b). The 4-kDa amphiphilic protein has a length of 39–43 amino acids with the following sequence:

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1                                   25
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL–
                                    35      43
                                    MVGGVVIAT
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It is generated by proteolytic cleavage of the transmembrane amyloid precursor protein (APP) (Kang et al., 1987). Like other known types of amyloid proteins, βAP aggregates into β-sheets, leading to the formation of insoluble fibrils. The β-sheet structure appears to vary among the different amyloid proteins and molecular details are known only for a few special cases (cf. Lansbury, 1992).

Studies on rat hippocampal neurons in culture have shown that the major βAP found in cerebrovascular amyloid deposits, i.e. βAP(1–40), and the fragment βAP(25–35) have the same early neurotrophic and late neurotoxic activities (Yankner et al., 1990). Furthermore, the peptide fragment βAP(25–35) exhibits sequence homologies with neuropeptides of the tachykinin family such as substance P, with a positively charged residue in the N-terminal region and a C-terminal domain constituted mainly by hydrophobic amino acids (Masters et al., 1985b; Yankner et al., 1990). The hydrophobic C-terminal of the tachykinin neuropeptides is required for high-affinity receptor binding and biological activity; however, the fragment βAP(25–35) does not interact with tachykinin receptors (Distefano et al., 1993; Burgevin et al., 1992; Lee et al., 1992). On the other hand, βAP(25–35)NH_2 has been reported to mimic the function of substance P on the modulation of the nicotinic response of cultured bovine chromaffin cells (Cheung et al., 1993), suggesting the possible involvement of the nicotinic receptor in Alzheimer's disease.

The assembly process of βAP and of some of its synthetic fragments has been under intense investigation since it might shed light on the mechanism of plaque formation (Barrow & Zagorski, 1991; Jarett et al., 1993; Pike et al., 1993). Kinetic studies suggested that amyloid formation, like crystallization, is a nucleation-dependent phenomenon and that the nucleation step is *kinetically* hindered (Jarett et al., 1993). Here we report data on the synthetic peptide βAP(25–35), located near the transmembrane region of βAP, which exhibits a reversible, concentration-dependent aggregation, similar to that observed for micelle formation. Aggregation is accompanied by a reversible random coil ⇄ β-sheet transition and can be described in terms of either a non-cooperative model or a cooperative model of moderate cooperativity. The random coil ⇄ β-sheet equilibrium can be modulated by pH and by small alterations in the chemical structure.

MATERIALS AND METHODS

Peptide Synthesis. βAP(25–35)OH was purchased from Saxon Biochemicals, Hannover, Germany. βAP(25–35)NH_2 and the Nle derivative βAP(25–35Nle)OH were synthesized on the 9050 peptide synthesizer of Milligen using the solid-phase peptide methodology. The former peptide was assembled on a polyacrylamide resin derivatized with a Rink linker (Rink, 1987) to obtain a C-terminal amide, whereas the latter peptide was assembled on the polyacrylamide resin directly. (9-Fluorenylmethoxy)carbonyl-protected (Fmoc-protected) amino acids were used in 4-fold excess in the coupling step with diisopropylcarbodiimide/*N*-hydroxylbenzotriazole as coupling reagent. The Fmoc protecting group was cleaved

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[1] Abbreviations: AcONa, sodium acetate; MOPS, morpholinopropanesulfonic acid; CD, circular dichroism; βAP, β-amyloid peptide; APP, β-amyloid precursor protein.

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with 20% piperidine in dimethylformamide. Cleavage from the resin was carried out with trifluoroacetic acid/anisole in both cases. Purification was done using chromatography on Sephadex G10 and preparative HPLC. The purity and the identity of the synthesized and purchased products were checked by analytical HPLC (better than 97% purity), NMR, FAB-MS, and amino acid analysis.

Circular Dichroism Spectroscopy. CD measurements were carried out on a Jasco J720 spectropolarimeter. All measurements were performed at room temperature. The path length of the quartz cell was either 1 or 0.1 mm. All spectra were corrected by subtracting the buffer baseline. Results are expressed in terms of mean residue ellipticity $[\theta]$ in units of deg cm^2 dmol^{-1}. The percentages of the secondary structures were calculated with the protein secondary structure estimation program SSE-338 purchased from Jasco. This program is based on reference spectra of Yang et al. (1986).

Sample Preparation. CD measurements were performed at pH 4.0 and 5.5 using 5 mM sodium acetate buffer and at pH 7.4 using 5 mM MOPS. Appropriate amounts of buffer were added to the dry peptides and were equilibrated with stirring for 1–2 h. The concentration of peptide in the stock solution was 0.5 or 1 mM. For βAP(25–35)OH which was the least soluble of the three peptides, a 0.5 mM stock solution represents an equilibrium between colloidal fibrils and truly dissolved monomers. Stock solutions were freshly prepared for each set of dilution experiments. By monitoring the diluted sample with CD, a new equilibrium state was usually observed within 30 min. After equilibration the CD spectrum remained unchanged for 24 h ($C_{pep} \leq 100$ μM) or at least a few hours (for higher peptide concentrations).

The peptide content of the stock solutions was determined by amino acid analysis.

Molecular Modeling. Modeling studies were performed using the Sybyl program package (Vers. 5.5. TRIPOS, St. Louis, MO). The starting structure was built using the ideal backbone torsion angles of an antiparallel β-pleated sheet for the whole sequence. Two molecules were adjusted to ensure the correct hydrogen bonding pattern for seven residues. The dimer was energy minimized to remove unfavorable interactions while keeping the hydrogen bonds and the salt bridge between the Lys-28 and Met-35 of opposite strands as constraints. The resulting structure must thus be considered as a local energy minimum.

Ultracentrifugation Experiments. Sedimentation equilibrium experiments were performed in a Beckman model XLA analytical ultracentrifuge. The 12-mm double-sector cell was filled with 0.12 mL solution in one sector and the same amount of solvent in the other (filling height ~3 mm). Molecular weights were calculated using linear regression analysis to obtain the best linear fit of ln (absorbance) vs r^2. A partial specific volume of 0.73 mL/g was used.

RESULTS

Conformational Studies of βAP(25–35)OH. βAP(25–35)-OH is characterized by a free carboxy terminus at Met-35. The peptide conformation in solution was measured at pH 4.0, 5.5, and 7.4. Figure 1 displays CD spectra of βAP(25–35)OH at pH 4.0 measured as a function of peptide concentration. As the spectra were obtained by dilution of a concentrated stock solution we discuss them in the order high concentration (curve 5) → low concentration (curve 1). At high peptide concentrations, the CD spectra exhibit a distinct minimum at 217 nm which is the characteristic signature of the β-sheet structure. Upon dilution, the intensity

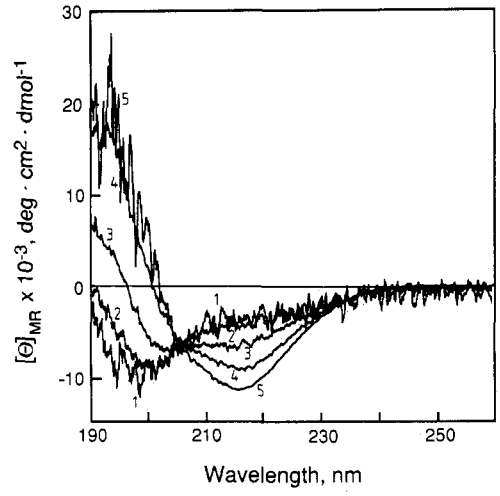

FIGURE 1: Circular dichroism spectra of β-amyloid peptide βAP-(25–35)OH at pH 4.0 (5 mM sodium acetate buffer): concentration-dependent β-sheet ⇄ random coil transition. Random coil structure is favored at high dilution. Curve assignments are as follows: 1, 31; 2, 62.5; 3, 125; 4, 250; 5, 500 μM.

of the 217-nm band is reduced and a new minimum develops at about 200 nm, indicating a predominantly random coil structure with some admixture of β-sheet structure. The β-sheet → random coil transition exhibits an *isodichroic point* at 206 nm which provides evidence for a simple two-state β-sheet ⇄ random coil equilibrium. This is consistent with the absence of any notable α-helix absorption at 208 and 222 nm.

Closer inspection of Figure 1 reveals a considerable increase in noise for the most concentrated solution (at short wavelength) indicative of enhanced light scattering. In order to detect the formation of peptide aggregates, the higher concentrated peptide solution (0.5 mM) was filtered through a Millipore filter of 0.22-μM pore size. The filtrate was measured immediately after filtration and showed a predominantly random coil CD spectrum. Electron microscopy of the solid material retained by the millipore filter revealed small fibers.

The percentage of secondary structure was estimated with the computer program of the JASCO 720 spectropolarimeter, and the results are summarized in Table 1. Since the program is based on reference spectra obtained for proteins which differ in structure from amyloid proteins, the quantitative analysis must be considered as a first approximation only. Nevertheless, two features of this automatic fitting routine are obvious, namely (i) the percentage of random coil increases and (ii) the percentage of β-sheet decreases by a factor of 5–6 as the peptide concentration decreases from 500 to 10 μM. The data thus demonstrate a *concentration-induced* β-sheet ⇄ random coil transition of βAP(25–35)OH.

Additional CD measurements on βAP(25–35)OH were performed at pH 5.5 and pH 7.4. At pH 5.5 the results were very similar to those obtained at pH 4.0: A concentration-dependent β-sheet ⇄ random coil transition was observed upon dilution of the stock solution (data not shown). In contrast, only a β-sheet structure was observed at pH 7.4, even at the lowest concentration measured (cf. Figure 2A). In addition, the most concentrated solution had a turbid appearance, indicating the formation of large peptide aggregates.

In the above dilution experiments CD spectra were measured typically 30 min after dilution of a freshly prepared stock solution. The CD spectra were unchanged when the same solutions were remeasured several hours later. However, a

Table 1. Conformational Analysis of the CD Spectra of βAP(25–35)OH as a Function of Peptide Concentration at pH = 4.0 in 5 mM AcONa Buffer

C_{pep} (μM)	% random coil	% β-sheet	% β-turn	% α-helix
10	63.5	11.2	25.3	0
15.6	57.1	16.6	23.6	2.8
20	52.1	30.4	17.5	0
30	41.0	33.7	25.3	0
30	54.1	30.3	15.5	0
31	47.5	26.6	21.8	4.1
40	48.7	33.5	14.5	3.3
50	48.1	37.7	11.9	2.3
60	45.3	33.2	11.2	9.6
62.5	43.7	39	15.2	2.1
70	39.7	42.1	6.1	12.1
100	36.6	50.7	9.5	3.2
100	33	48.9	12.2	5.9
100	33.8	52.7	8.3	5.2
125	35.8	43.8	13.6	6.7
125	19.1	60.0	3.5	17.5
175	17.6	63.6	0.7	18.1
200	11.7	62.3	3.2	22.8
250	20.7	60.5	2.6	16.2
250	25.7	56.8	6.9	10.6
250	23.3	55	7.1	14.6
500	13.8	67.4	0	18.8
500	23	58.5	6.3	12.2
500	6.1	62.7	0	31.2
500	22.4	61	4.5	12.1

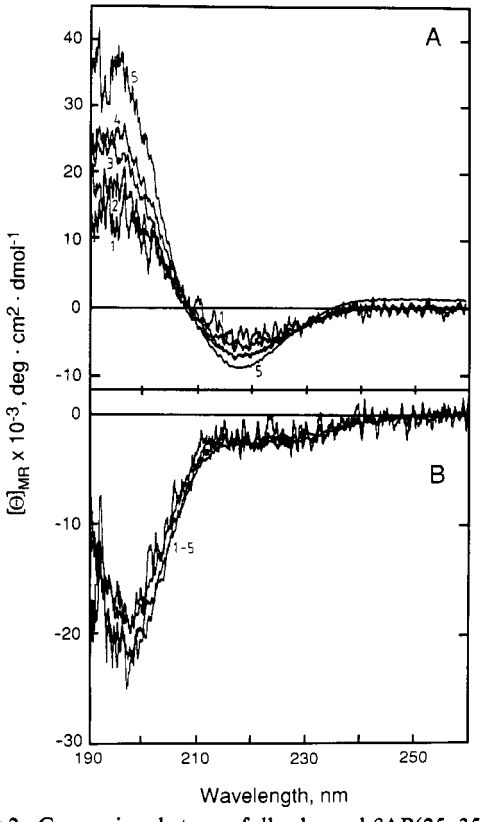

FIGURE 2: Comparison between fully charged βAP(25–35)OH and amidated βAP(25–35)NH_2. Part A shows CD spectra of βAP(25–35)OH at pH 7.4 (5 mM MOPS). Independent of peptide concentration the peptide adopts predominantly a *β-sheet structure*. Curve assignments are as follows: 1, 31; 2, 63; 3, 100; 4, 250; 5, 375 μM. Part B shows CD spectra of βAP(25–35)NH_2 at pH 4.0 (5 mM sodium acetate). The CD spectra are characteristic of a predominantly *random coil structure* at all concentrations. Curve assignments are as follows: 1, 35 μM; 2, 140 μM; 3, 333 μM; 4, 667 μM; 5, 1 mM.

different situation was encountered when the starting solution contained monomers only. This was achieved experimentally by separating fibrils from monomers using Millipore filters (0.22-μm diameter). For a solution with an initial peptide concentration of C_{pep} = 500 μM (at pH = 4), the corresponding filtrate revealed a random coil CD spectrum with a peptide concentration of $C_{pep} \approx 129$ μM, indicating a loss of 74% aggregated (β-sheet) peptide via filtration. For a 100 μM solution, the filtrate had a concentration of ~45 μM and the loss was only 55%. Interestingly, the CD spectra of the filtrates revealed a random coil structure even 48 h after filtration. Apparently, the formation of new aggregates was kinetically hindered. Kinetic hindrance could however be relieved by seeding the solution with a few fibrils in agreement with recent experiments on related βAPs and βAP fragments (Jarrett et al., 1993). Equilibration between random coil monomers and β-sheet fibrils was then established fairly rapidly as monitored with CD spectroscopy.

The monomeric state of βAP(25–35)OH in the filtered solutions was demonstrated with ultracentrifugation experiments. A 0.5 mM solution of βAP(25–35)OH in buffer (5 mM NaOAc, pH 4.0) was filtered through a 0.22-μm Millipore filter and the filtrate centrifuged for 6 h at 56 000 rpm on a Beckman XLA analytical ultracentrifuge (An-60 Ti analytical rotor, 20 °C). The analysis of the sedimentation equilibrium (at 222 nm) yielded a molecular weight of 1150 ± 35 Da (2 runs), in good agreement with the theoretical molecular weight of 1060.4 Da for βAP(25–35)OH monomers. No aggregates were detected. In contrast, when the measurement was repeated with an unfiltered sample, sedimentation of larger aggregates was observed starting at 10 000 rpm. Centrifugation was continued at 24 000 rpm until the aggregates reached the bottom. Increasing the rotational speed to 56 000 rpm led to the formation of a sedimentation equilibrium pattern characteristic of monomers only.

Conformational Studies of βAP(25–35)NH_2 and βAP(25–35Nle)OH. Peptide βAP(25–35)OH was modified by (i) substituting the carboxylate function by an amide function or (ii) by replacing methionine at position 35 by norleucine. For both peptides rather similar CD spectra were recorded at all concentrations and pH values. Figure 2B summarizes representative results for βAP(25–35)NH_2 at pH 4.0. The spectra are predominantly random coil (>60%) with some admixture of β-turn and β-sheet. For the norleucine derivative (data not shown), the percentage of random coil was reduced to 50% with a concomitant increase in β-turn and β-sheet. A reversible β-sheet ⇄ random coil transition was not observed. Changing the pH to 5.5 or 7.4 had no effect on the CD spectra of both peptides.

DISCUSSION

Human β-amyloid protein is a 39–43 amino acid polypeptide which is rather insoluble under physiological conditions. It has been proposed that the first 28 residues constitute an extracellular domain whereas residues 29–40 (43) are anchoring the peptide in the lipid membrane (cf. Fraser et al., 1991). The synthetic peptide βAP(25–35) overlaps partly with both domains. Lipid-binding studies revealed however no strong binding of βAP(25–35) to electrically neutral lipid bilayers (Terzi, E., Hölzemann, G., & Seelig, J., manuscript in preparation).

The most interesting property of βAP(25–35)OH is its reversible β-sheet ⇄ random coil transition at pH 4.0 and 5.5. Concentrated solutions of βAP(25–35)OH contain microfibrils with β-structure which upon dilution disintegrate into random coil monomers. Fibril formation from a purely monomeric solution appears to be kinetically hindered but can be catalyzed by seeding with βAP(25–35)OH fibrils.

Human βAP(1–42) when dissolved in buffer containing 32.5% trifluoroethanol also exhibits a CD spectrum characteristic of β-sheet structure. However, dilution studies had practically no effect on the appearance of the CD spectrum (Otvos et al., 1993). In contrast, a β-sheet ⇄ random coil transition was observed for the related *rodent* βAP(1–42) which differs from *human* βAP(1–42) in three amino acid substitutions (Otvos et al., 1993). The dilution studies were again performed with a solvent containing 32.5% trifluoroethanol. TFE increases the solubility of the peptide and, at the same time, is thought to mimic the hydrophobic membrane environment.

Different fragments of βAP(1–42) have also been synthesized in order to elucidate the structural determinants involved in plaque formation. Both the proposed membrane-anchoring domain βAP(29–42) and the extracellular domain βAP(1–28) exhibit β-sheet and fibril formation in a narrow range of conditions (Halverson et al., 1990, 1991; Fraser et al., 1991; Barrow et al., 1992). None of these earlier studies on βAP fragments revealed a simple two-state equilibrium. We are also not aware of any quantitative treatment of the assembly process. Due to the reversibility of random coil ⇄ β-sheet transition of βAP(25–35)OH, a simple thermodynamic analysis can now be given.

Thermodynamic Analysis. The CD spectra of βAP(25–35)OH measured as a function of concentration at pH 4.0 (Figure 1) and pH 5.5 showed an isodichroic point, which is consistent with the presence of just two conformations or two molecular species, i.e. monomers in the random coil conformation and oligomers with predominantly β-sheet structure. The concentration-dependent self-association can be described by a simple model which has been employed successfully in other aggregation processes of biomolecules (cf. Robinson et al., 1975; Heyn & Bretz, 1975; Cantor & Schimmel, 1980, p 145). The formation of a dimer is considered as the *nucleation* step

$$A + A \overset{\sigma s}{\rightleftarrows} A_2 \qquad C_{A_2} = (\sigma s) C_A^{\,2} \tag{1}$$

which is characterized by the equilibrium constant σs. σ is denoted *nucleation parameter*. Fibers are formed by continuous addition of monomers according to the sequence

$$A_2 + A \overset{s}{\rightleftarrows} A_3 \qquad C_{A_3} = sC_{A_2}C_A = \sigma s^2 C_A^{\,3} \tag{2}$$

$$A_{n-1} + A \overset{s}{\rightleftarrows} A_n \qquad C_{A_n} = sC_{A_{n-1}}C_A = \sigma s^{n-1} C_A^{\,n} \tag{3}$$

For the growth of the oligomers via the addition of monomers a constant *growth parameter* s was used. The total concentration of peptide, C_o, expressed in monomer units is given by

$$C_o = C_A + 2C_{A_2} + 3C_{A_3} + \ldots + nC_{A_n}$$

$$C_o = C_A + 2\sigma s C_A^{\,2} + \ldots + n\sigma s^{n-1} C_A^{\,n}$$

$$C_o = C_A\{1 + \sigma(2sC_A + 3s^2 C_A^{\,2} + \ldots + ns^{n-1} C_A^{\,n-1})\} \tag{4}$$

The evaluation of the latter expression is straightforward with the assumptions of $n \to \infty$ and $sC_A < 1$ leading to

$$C_o = C_A\left\{1 - \sigma + \frac{\sigma}{(1 - sC_A)^2}\right\} \tag{5}$$

For a non-cooperative model, i.e. $\sigma = 1$, eq 5 is further simplified to

$$C_o = C_A/(1 - sC_A)^2 \tag{6}$$

For a given set of parameters σ, s, and C_o eqs 5 and 6 allow the evaluation of the monomer concentration C_A. The concentration of monomers forming aggregates is then given by $C_\beta = C_o - C_A$ and the *mole fraction* of monomers involved in β-structured fibers is found as

$$X_\beta = (C_o - C_A)/C_o \tag{7}$$

Table 1 summarizes the quantitative evaluation of the CD spectra of βAP(25–35)OH in terms of percentages of random coil, β-turn, and β-sheet structures. Molecules with β-sheet structure are involved in fibril formation, whereas those adopting a random coil or β-turn structure are assumed to occur as monomers only. The mole fraction of aggregated monomers involved in fibril formation, denoted X_β in the following, can be calculated as

$$X_\beta = \frac{\%\ \beta\text{-structure observed}}{\text{maximum }\%\ \beta\text{-structure}}$$

For the maximum amount of β-structure we assumed 70% β-structure (which is close to the experimental result observed at the highest peptide concentration). Figure 3 displays the calculated X_β values and also the best theoretical fits using the cooperative and the non-cooperative models. The theory suggests a cooperativity parameter of $\sigma = 0.2$ and a binding constant of $s = (2.9 \pm 1.2) \times 10^4\ M^{-1}$ for the cooperative model and $s = (1.8 \pm 1) \times 10^4\ M^{-1}$ for the non-cooperative model ($\sigma = 1$). The free energy of association is $\Delta G \approx -6.0$ kcal/mol for both models. In addition, within the accuracy of the measurement, the data obtained at pH 5.5 lead to the same thermodynamic parameters as those measured at pH 4.0.

In order to estimate the enthalpy of association, ΔH_a, dilution experiments were performed using titration calorimetry (E. Terzi, G. Hölzemann, and J. Seelig, manuscript in preparation). A concentrated solution of βAP(25–35)OH was injected into a pure buffer solution. An *endothermic* dissociation reaction of about +3 kcal/mol was observed, i.e. the association reaction is *exothermic* by the same amount. The enthalpy of association, $\Delta H_a \approx -3$ kcal/mol and thus accounts for at least 50% of the free energy of aggregation.

The carboxy terminus of βAP(25–35)OH appears to play an important role in β-sheet formation. If the carboxylic group is *fully* charged (pH 7.4) the CD spectra reflect maximum β-structure at all concentrations (Figure 2A). For a *partially* charged carboxy terminus (pH 4) a concentration-dependent β-sheet ⇄ random coil transition is observed. *Elimination* of charge by amidation of the carboxy terminus leads to a predominantly random coil structure (Figure 2B). A possible interpretation of these results is suggested by Figure 4 which depicts two molecules of βAP(25–35) aligned in an antiparallel β-pleated sheet. The dimer was energy minimized to remove all unfavorable interactions thus showing a local energy minimum structure (cf. Materials and Methods).

The characteristic features of this model are two intermolecular ion pairs between Lys-28 and Met-35, with 3 amino acids of each peptide dangling at the end of the β-sheet. This model suggests a maximum of 70% β-structure, in agreement with the experimental observations. Ionic interactions then appear to play a central role in the stability of this β-sheet. Protonation of the carboxyl group or removal of its electric charge by amidation shifts the equilibrium toward the random coil monomeric state. However, if Met-35 is replaced by Nle,

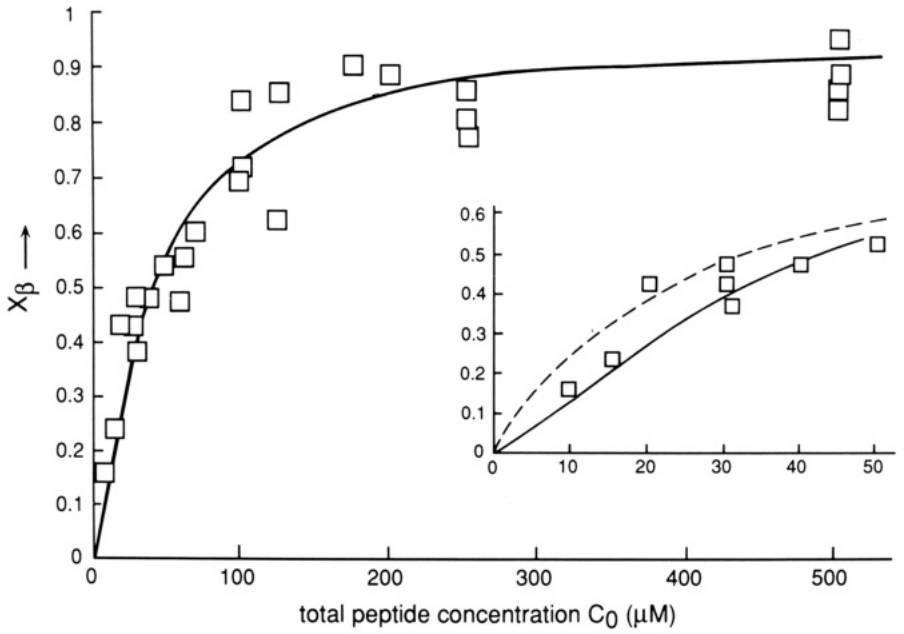

FIGURE 3: Concentration-dependent random coil ⇄ β-sheet transition of βAP(25–35)OH at pH 4.0. The mole fraction of β-sheet, X_β (i.e. the mole fraction of monomers involved in β-sheet formation), is plotted versus the total monomer concentration. The experimental results are compared with a cooperative model (—, $\sigma = 0.2$; $s = 2.9 \times 10^4$ M^{-1}) and a non-cooperative model (- - -, $\sigma = 1$; $s = 1.8 \times 10^4$ M^{-1}). The difference between the two models is most pronounced in the low concentration range.

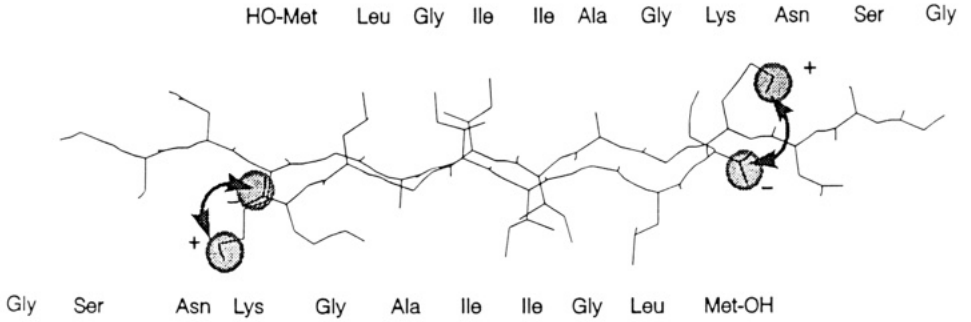

FIGURE 4: Molecular model of β-sheet formation by βAP(25–35)OH. Two molecules of βAP(25–35)OH are aligned antiparallel to each other. Ion pair interactions stabilize the two ends of the β-pleated sheet structure.

the amidated derivative (βAP(25–35Nle)NH_2) undergoes a concentration-dependent random coil ⇄ β-structured aggregate transition (E. Terzi, G. Hölzemann, and J. Seelig, manuscript in preparation). Nle is a well-known β-structure promoter and this result suggests that the ionic interaction Lys-28···Met-35 is not an absolute requirement for β-sheet formation but can be replaced by a strong van der Waals interaction.

βAP(1–43) and some of its fragments can adopt quite different conformations depending on the solution conditions (cf. Barrow et al., 1992). Using solvent mixtures with fluorinated alcohols such as trifluoroethanol it has been possible, for example, to induce an α-helix ⇄ β-sheet transition in the βAP(1–42) and βAP(1–39) peptides (Barrow et al., 1992). It has further been demonstrated that fibril assembly and disassembly are pH-dependent processes, with β-structure formation being favored at pH 4 to 7 (Fraser et al., 1991; Burdick et al., 1992; Barrow & Zagorski, 1991, Barrow et al., 1992). The present study then appears to be the first quantitative description of a reversible, concentration-induced random coil ⇄ β-sheet equilibrium. Moreover, fragment βAP-(25–35)OH is one of the shortest peptide molecules observed so far to exhibit the formation of large β-structured fibrils. Segment βAP(25–35)OH could thus be involved in the initial interactions leading to the aggregation of the full-length peptide βAP(1–43) in amyloid-like deposits.

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