# Peptide Models of a Hydrophobic Cluster at the C-Terminus of the β-Amyloid Protein†

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*Received June 27, 1994*

The folding of soluble proteins may be initiated by the formation of hydrophobic clusters comprising the side chains of residues which are close in primary sequence.[1-3] Similarly, protein aggregation to form insoluble amyloid may be initiated by a soluble, monomeric hydrophobic cluster. Since amyloid fibril formation follows a nucleation-dependent mechanism,[4] a small change in the concentration of the aggregating species can have a dramatic effect on the rate of aggregation. For example, a 10% increase in its concentration will increase the rate of formation of a 20-mer nucleus by $(1.1)^{20}$, or nearly 7-fold.[4]

An unusual Gly−Gly cis amide bond may characterize the amyloid fibril comprising a peptide (β34−42) based on the C-terminus of the Alzheimer's disease (AD) amyloid plaque protein (β-protein, β1−42, Figure 1).[5] This local conformation could be stabilized in solution by a hydrophobic cluster involving the flanking residues. We report herein solution $^1$H NMR studies which demonstrate that peptide models of the β-protein C-terminus form soluble hydrophobic clusters containing a cis amide bond. Stabilization of the cis amide by cluster formation suggests a possible explanation for the higher rate of amyloidogenesis observed for the "long" β-proteins (β1−42 and β1−43) relative to "short" β (β1−40).[6]

A series of peptides based on the β-protein C-terminus were synthesized in order to probe the effect of the flanking hydrophobic sequence on the amide cis/trans ratio. The equilibrium fraction of the cis amide conformer is too small to quantify by solution $^1$H NMR spectroscopy (<2%, $\Delta G$ > 2 kcal/mol).[7-9] Therefore, the Gly37−Gly38 amide bond was replaced with an *N*-methylamide (Gly38 = sarcosine, Sar), which affords comparable populations of cis (*E*) and trans (*Z*) conformers in solution (Table 1, entry **1**).[10] The cis and trans *N*-methyl groups are well-resolved singlets in the $^1$H NMR spectrum, the integration of which provides an accurate measure of the cis/trans ratio.

1 34
$H_2N$-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-$CO_2H$ β1-40

$H_2N$-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-$CO_2H$ β1-42

**Figure 1.** Amino acid sequences of two β-protein variants. β1−40 is the normal circulating variant, whereas β1−42 is the major AD amyloid plaque protein.[19,20] The highlighted sequence (Gly37−Gly38) may be characterized by a cis amide bond in the β34−42 amyloid fibril.[5b]

The results of the $^1$H NMR studies are summarized in Table 1.[11] In general, increasing the hydrophobicity of the residues flanking the *N*-methylamide led to an increased percentage of cis conformer (i.e., **1** → **2** → **3** → **4** → **5**). Addition of Val40 (**4** → **6** and **8** → **9**) increased the amount of cis isomer by 6%,[15] suggesting that Val36, Val39, and Val40 are involved in a stabilizing hydrophobic interaction (Figure 2). However, addition of Met35 (**4** → **8** and **6** → **9**) had only a small effect in this context. No cooperative effect between Met35 and Val40 was observed (i.e., **4** → **6** = **8** → **9** and **4** → **8** = **6** → **9**), indicating that Met35 does not interact with Val39 or Val40. The amount of cis isomer observed in peptides **4** and **6** increased in saturated NaCl solution and decreased to the level of peptide **1** in 6 M guanidinium thiocyanate (peptide **1** showed no significant change).[16] This is consistent with a hydrophobic stabilization of the cis conformation.[17] Examination of molecular models suggests that hydrophobic cluster formation by minimization of the solvent-accessible surfaces of Val36 and Val39 requires a cis amide at Gly37−Gly38.

Addition of Leu34 and/or Ile41 to the models resulted in peptides which were not soluble enough to be accurately analyzed. Therefore, peptides **9**−**12**, in which Met35 and Val40 were replaced with glutamic acid, were synthesized and analyzed. These models allowed assessment of the effect of Ile41, which distinguishes the normal circulating variant of the β-protein (β1−40 or short β)[18,19] from the rapidly aggregating,

† This paper is dedicated to Professor Glenn Berchtold, on the occasion of his retirement from this department.

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(1) (a) Matheson, R. R., Jr.; Scheraga, H. A. *Macromolecules* **1978**, *11*, 819. (b) Rose, G. D.; Roy, S. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4643. (c) Wright, P. E.; Dyson, H. J.; Lerner, R. A. *Biochemistry* **1988**, *27*, 7167. (d) Garvey, E. P.; Swank, J.; Matthews, C. R. *Proteins* **1989**, *6*, 259. (e) Chan, H. S.; Dill, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6388. (f) Wiley, R. A.; Rich, D. A. *Med. Res. Rev.* **1993**, *13*, 327. (g) Dill, K. A.; Fiebig, K. M.; Chan, H. S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1942. (h) Simmerling, C.; Elber, R. *J. Am. Chem. Soc.* **1994**, *116*, 2534. (i) Gronenborn, A. M.; Clore, G. M. *Science* **1994**, *263*, 536.

(2) Hydrophobic clusters have been observed at high denaturant concentration: (a) Neri, D.; Billeter, M.; Wider, G.; Wüthrich, K. *Science* **1992**, *257*, 1559. (b) Evans, P. E.; Topping, K. D.; Woolfson, D. N.; Dobson, C. M. *Proteins* **1991**, *9*, 246. These clusters, as well as the one modeled herein, do not necessarily persist in the final protein.

(3) Artificial folding templates can act by stabilizing hydrophobic clusters: (a) Díaz, H.; Tsang, K. Y.; Choo, D.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 3790. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988.

(4) Jarrett, J. T.; Lansbury, P. T., Jr. *Cell* **1993**, *73*, 1055.

(5) (a) Halverson, K.; Fraser, P. E.; Kirschner, D. A.; Lansbury, P. T., Jr. *Biochemistry* **1990**, *29*, 2639. (b) Spencer, R. G. S.; Halverson, K. J.; Auger, M.; McDermott, A. E.; Griffin, R. E.; Lansbury, P. T., Jr. *Biochemistry* **1991**, *30*, 10382.

(6) Jarrett, J. T.; Berger, E. P.; Lansbury, P. T., Jr. *Biochemistry* **1993**, *32*, 4693.

(7) Ramachandran, G.; Mitra, A. *J. Mol. Biol.* **1976**, *107*, 85.

(8) Herzberg, O.; Moult, J. *Proteins* **1991**, *11*, 223.

(9) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253.

(10) The effect of flanking residues on the Gly−Sar cis/trans ratio should be proportional to the effect on the Gly−Gly ratio. Examination of models suggests that the Sar *N*-methyl group cannot directly interact with neighboring side chains to stabilize the cis conformation. In addition, NOE difference and ROESY spectra of **4** show no measurable interaction between the *N*-methyl group and any of the Val36 or Val39 protons.

(11) Peptides were synthesized on the 4-methylbenzhydrylamine (MBHA) resin using BOP reagent[12] for couplings (except for the Gly to Sar coupling, for which PyBrOP[13] was used), cleaved with trifluoromethanesulfonic acid (TFMSA),[14] purified by reverse phase HPLC and characterized by plasma desorption mass spectrometry and $^1$H NMR. Solutions of peptides in aqueous buffer (100 mM NaCl, 10 mM $NaH_2PO_4$, pH 7.4) were prepared at *ca.* 10 mM. $^1$H NMR spectra were recorded on a Varian Unity 500 MHz NMR spectrometer. Cis/trans ratios shown are an average of at least 10 separate integrations of the *N*-methyl proton singlets (standard deviation ≤ 0.3%). No differences in cis/trans ratio were observed over a concentration range of 3−30 mM.

(12) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.

(13) Coste, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669.

(14) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984.

(15) Percent changes in cis amide population cited in the text are calculated relative to the initial population of cis: % increase = % change/% $cis_{initial}$.

(16) Cis content (%) for peptides **1**, **4**, and **6** (saturated NaCl, 6 M GdnSCN): **1**, 23.3, 22.3; **4**, 27.8, 22.7; **6**, 26.6, 23.8.

(17) The following thermodynamic parameters were calculated from a van't Hoff analysis of peptides **4** and **9** over the range 10 °C to 50 °C (10 mM peptide, 100 mM NaCl, 10 mM $NaH_2PO_4$, pH 7.4). For peptide **4**, $\Delta H$(trans → cis) = +0.325 kcal/mol (±0.1); $\Delta S$(trans → cis) = −1.1 eu (±0.4). For peptide **9**, $\Delta H$(trans → cis) = +0.327 kcal/mol (±0.1); $\Delta S$(trans → cis) = −0.8 eu (±0.4). The entropy change is difficult to interpret, since solvent structure must be considered. The difference in heat capacity, which has been used to assess solvent ordering around denatured proteins, was not expected to be interpretable in the case of the small, multiconformational peptides discussed herein and was, therefore, not measured.[2a]

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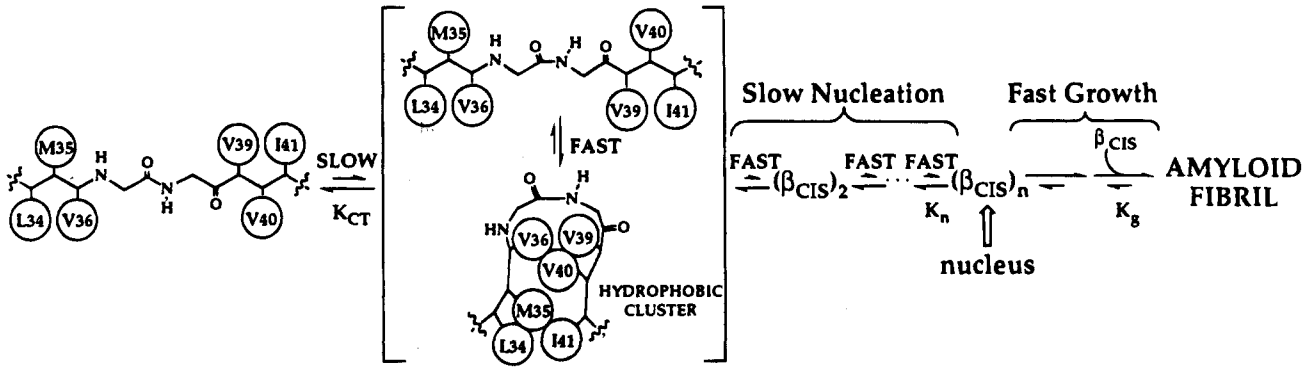


**Figure 2.** One possible mechanism for AD amyloid fibril formation. Initial trans to cis isomerization is slow relative to cluster formation and the subsequent intermolecular association processes.[21] Therefore, $K_{CT}$ is a critical determinant of the rate of nucleus formation. The cis amide is stabilized by formation of a hydrophobic cluster. The side-chain interactions indicated were inferred from the model studies and may be direct or indirect. They do not necessarily persist in the amyloid fibril.[2]

**Table 1.** Cis Amide Population in β-Amyloid C-Terminal Model Peptides

| peptide | cis content, % (±0.3%) |
|---|---|
| **1**, Ac-Gly-Sar-$CONH_2$ | 22.3 |
| **2**, Ac-Ala-Gly-Sar-Ala-$CONH_2$ | 22.6 |
| **3**, Ac-Ala-Gly-Sar-Val-$CONH_2$ | 24.3 |
| **4**, Ac-Val-Gly-Sar-Val-$CONH_2$ | 25.0 |
| **5**, Ac-Ile-Gly-Sar-Ile-$CONH_2$ | 25.4 |
| **6**, Ac-Val-Gly-Sar-Val-Val-$CONH_2$ | 26.6 |
| **7**, Ac-Val-Gly-Sar-Val-Val-Ile-$CONH_2$ | 26.6 |
| **8**, Ac-Met-Val-Gly-Sar-Val-$CONH_2$ | 25.3 |
| **9**, Ac-Met-Val-Gly-Sar-Val-Val-$CONH_2$ | 26.9 |
| **10**, Ac-Glu-Val-Gly-Sar-Val-Glu-$CONH_2$ | 24.9 |
| **11**, Ac-Leu-Glu-Val-Gly-Sar-Val-Glu-$CONH_2$ | 24.9 |
| **12**, Ac-Glu-Val-Gly-Sar-Val-Glu-Ile-$CONH_2$ | 25.7 |
| **13**, Ac-Leu-Glu-Val-Gly-Sar-Val-Glu-Ile-$CONH_2$ | 26.4 |

plaque β-proteins (β1−42 and β1−43, long β),[6,20] Ile41 appears to interact with both Leu34 (**11** → **13** = +6%) and Glu(Met)-35 (**10** → **12** = +3%) but not residues 36, 39, and 40 (**6** → **7** = 0%) (Figure 2).

The hydrophobic cluster modeled herein could accelerate aggregation of β1−42 by stabilizing the slowly isomerizing cis amide at Gly37−Gly38.[21] However, the kinetically unstable features of the cluster (side-chain hydrophobic interactions) do not necessarily persist in the amyloid fibril (Figure 2).

The long β-protein β1−42, the primary constituent of AD amyloid plaque,[20b] nucleates much more rapidly than β1−40.[6,22] It has been suggested that β1−42 is the pathogenic variant and that its overproduction may induce early onset AD.[6,22,23] In the model system, increases of about 6% in the equilibrium cis amide population were observed for addition of Val40 and Ile41. Given a reasonable nucleus size of 25, such an increase would lead to a greater than 4-fold $\{(1.06)^{25}\}$ acceleration of nucleation, providing a partial explanation for the difference between long β and short β.[6,22] It may therefore be possible to inhibit amyloid formation by preventing hydrophobic cluster formation at the β-protein C-terminus.

**Acknowledgment.** We thank Stephen Younkin for keeping us abreast of unpublished results from his laboratory.[20] This work was supported by the National Institutes of Health (AG08470), the Camille and Henry Dreyfus Foundation, the Sloan Foundation, and the National Science Foundation (Presidential Young Investigator Award; contributions from Parke-Davis, Monsanto, Hoechst-Celanese, Merck, Abbott, Genentech, and Upjohn). P.T.L. is the Firmenich Career Development Professor of Chemistry. P.H.W. is an American Chemical Society Division of Medicinal Chemistry Fellow (funded by Eli Lilly & Co.). J.T.J. was an NIH predoctoral trainee (1T32GM08318) during a portion of this work.

(18) For a review, see: Selkoe, D. J. *Neuron* **1991**, *6*, 487.

(19) (a) Mori, H.; Takio, K.; Ogawara, M.; Selkoe, D. J. *J. Biol. Chem.* **1992**, *267*, 17082. (b) Miller, D. L.; Papayannopoulos, I. A.; Styles, J.; Bobin, S. A.; Lin, Y. Y.; Biemann, K.; Iqbal, K. *Arch. Biochem. Biophys.* **1993**, *301*, 41. (c) Dovey, H. F.; Suomensaari-Chrysler, S.; Lieberburg, I.; Sinha, S.; Keim, P. S. *NeuroReport* **1993**, *4*, 1039. (d) Vigo-Pelfrey, C.; Lee, D.; Keim, P.; Lieberburg, I.; Schenk, D. B. *J. Neurochem.* **1993**, *61*, 1965.

(20) (a) Cheung, T. T.; Suzuki, N.; Cai, X.-D.; Odaka, A.; Otvos, L., Jr.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336. Younkin has recently demonstrated that AD plaques contain as much as 95−99% long β: (b) Gravina, S. A.; Ho, L.; Suzuki, N.; Eckman, C. B.; Otvos, L., Jr.; Younkin, L. H.; Perry, G. R.; Younkin, S. G., unpublished results.

(21) In order for the cis−trans equilibrium constant ($K_{CT}$, Figure 2) to affect the rate of nucleation, isomerization must be slower than protein association. At the peptide concentration used in our aggregation assay (40 μM),[6] representative rates of ~10 $s^{-1}$ for interprotein association ($k \approx 10^5-10^6$ $M^{-1}$ $s^{-1}$)[24] and ~$10^{-2}$ $s^{-1}$ for amide isomerization[9] would be expected.

(22) The following nucleation times were observed for β-amyloid variants: β26−39, 60 h; β26−40, 12 h; β26−42 and β26−43, <15 s; β1−39, 96 h; β1−42, <15 s.[6]

(23) Cai, X.-D.; Golde, T. E.; Younkin, S. G. *Science* **1993**, *259*, 514.

(24) Koren, R.; Hammes, G. G. *Biochemistry* **1976**, *15*, 1165.