Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 369–371, January 1995
Neurobiology

Solubilization of β-amyloid-(1–42)-peptide: Reversing the β-sheet conformation induced by aluminum with silicates

(conformational changes/circular dichroism)

G. D. FASMAN*†, A. PERCZEL‡, AND C. D. MOORE*

*Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254; and ‡Department of Organic Chemistry, Eötvös University, Budapest, Hungary

Contributed by G. D. Fasman, September 26, 1994

ABSTRACT **Plaques are one of the two lesions found in the brain of patients with Alzheimer disease. Using a synthetic peptide corresponding to rat β-amyloid-(1–42) (βA4), circular dichroism (CD) analyses were performed to examine the effect of Na_4SiO_4 on the conformational state produced by Al^{3+}. A previous study on fragments of neuronal proteins involved in tangle formation had shown a conformational transition from a β-pleated sheet to a soluble random coil upon addition of Na_4SiO_4. In the present study, CD measurements showed that the β-pleated sheet conformation of βA4 induced by Al^{3+} was reversed to the random coil soluble form by the addition of Na_4SiO_4. The tight binding of SiO_4^{4-} with Al^{3+} provides the mechanism for this transition. These results provide insight into the role of aluminum in the Alzheimer diseased brain and suggests the investigation of the use of silicates as a therapeutic agent.**

Alzheimer disease (AD) will probably become the predominant social–medical–economic problem in the next century. An estimated 4 million Americans now have the disease, and this number is expected to grow to >10 million within the next decade (1). The cause of AD is not known, and treatments are only mildly effective at slowing the degeneration process (2). The signs of AD, obtained upon autopsy of the diseased brain, reveal two phenomena: amyloid plaques and neurofibrillary tangles (3). The amyloid plaques are composed primarily of a 39- to 42-amino acid fragment (βA4) from the amyloid precursor protein (4). Tangles contain abnormally phosphorylated tau protein that combine to form paired helical filaments within the neuron (5). In addition to the protein components of plaques and tangles, some researchers have detected aluminum (6, 7) and aluminosilicates (8) in these lesions, although the evidence for their presence is apparently controversial (9).

Aluminum is known to have toxic effects (10) on many organisms, including man. Aluminum is an associated risk factor in amyotrophic lateral sclerosis, Parkinsonism–dementia of Guam, and AD (11, 12).

Aluminum is the third most abundant element in the earth's crust, found primarily as insoluble aluminosilicates and oxides. However, it is soluble as aluminum hydroxide and aluminum hydrates at acidic pH values (13). This has become a biologic concern as the world's water supplies become more acidic due to acid rain. For example, Birchall *et al.* (14) have shown that in acidified water (pH 5), aluminum causes damage to gill epithelia and ultimate death for fish. The toxic effect of aluminum is diminished in the presence of silicic acid as the result of forming hydroxyaluminosilicates.

Silicon is the second most abundant element in the earth's crust and is present in rocks and minerals as SiO_2. It is soluble as $Si(OH)_4$. Implicated as a biologically important element, silicon has been shown in nutrition studies to be vital for growth and development (15). It has been detected in various human tissues. Silicon provides other beneficial effects in the form of silicic acid as it binds to aluminum (16); in addition to the results of Birchall *et al.* (14) mentioned above, silicon has been shown to reduce aluminum accretion in rat brains (17).

Model neurofibrillary tangle peptides of 17 amino acid residues (NF-M17) with and without phosphates on Ser^6 and Ser^{11} have been shown by circular dichroism (CD) studies to acquire the β-pleated-sheet conformation upon titration with Al^{3+}, before the Al^{3+} causes the peptide to precipitate (18, 19). The formation of β-pleated sheets could be reversed by the addition of silicates (20).

The current paper is a study of the βA4 peptide (residues 1–42 of the β-amyloid precursor protein), whose prominent role in the progression of AD has been frequently stressed and has been reviewed extensively (21–23). Several structural studies on βA4 or its fragments have been reported (24–28). CD studies illustrated the requirements for β-sheet filament formation (29). Solid-state Fourier-transform infrared spectroscopy showed that the secondary structure consisted of a β-turn flanked by two strands of antiparallel β-pleated sheet (30). In aqueous trifluoroethanol solutions, synthetic and naturally occurring β-amyloid peptides comprising residues 1–42 [β-(1–42)] and 1–39 [β-(1–39)] formed monomeric α-helical structures at high and low pH, as determined by NMR and CD; however, a monomeric β-structure predominated at pH 4–7 (25, 26). The kinetics of aggregation of the synthetic β-(1–40) peptide was followed and found to yield two-thirds β-structure but no α-helical structure (27), while a previous study had postulated that βA4 aggregates as β-sheets (30). In addition, it has been reported that aluminum promotes the β-sheet conformation of β-(1–40) (31) and its aggregation *in vitro* (32). Furthermore, the secondary structure of βA4—namely, its β-sheet conformation—was necessary to elicit neurotoxicity in three different assays using rat embryonic neuronal cell cultures (28). These results and others (see reviews in refs. 20–22) support a hypothesis concerning a role of the β-sheet conformation of βA4 and its involvement in the neurodegenerative process of AD. A reversible random coil–β-sheet transition has been demonstrated for the β-(25–35) fragment in aqueous medium (29). The present paper illustrates that the βA4 β-sheet conformation induced by Al^{3+} (models of plaques in AD) can be reversed or prevented by silicates. A similar experimental approach was previously used with model neurofilament peptides (model tangle peptides) (20).

The titration of βA4 (rat) with Al^{3+} and its reversal with SiO_4^{4-} was monitored by far-UV CD to detect any secondary structural changes; these spectra are shown in Fig. 1. Each spectrum was collected between 185 and 260 nm in 0.2-nm increments, time averaging each step for 5 s, and averaging two scans per titration point. At least 45 min passed between the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AD, Alzheimer disease; βA4, β-amyloid-(1–42)-peptide.
†To whom reprints requests should be addressed.

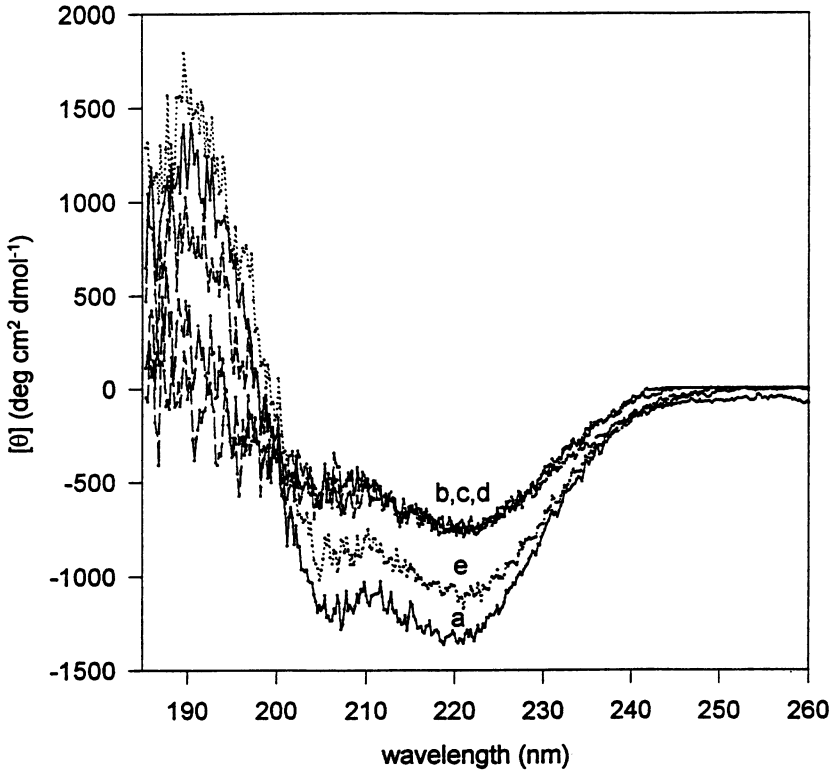

FIG. 1. βA4 peptide (residues 1–42) was synthesized by a solid-state synthetic method (35) using a fluorenylmethoxycarbonyl N-terminal protection strategy and a modified methylbenzhydrylamine resin. The peptide was purified by HPLC and characterized by amino acid analysis and positive-ion fast-atom bombardment mass spectroscopy. The CD titrations were performed in 2,2,2-trifluoroethanol (NMR grade). $Al(ClO_4)_3 \cdot 9H_2O$ was purchased from Aldrich; Na_4SiO_4 was obtained from Johnson Matthey Catalog Sales (Water Hill, MA). CD spectra were acquired with a Jobin-Yvon Mark V (Longjumeau, France) circular dichrograph. The CD titration of βA4 was performed with the peptide at 0.39 mg/ml in trifluoroethanol. The Al^{3+} solution (41.6 mM) was prepared in trifluoroethanol and Na_4SiO_4 was dissolved (138 mM) in water. Spectrum a (initial spectrum), peptide only; spectrum b, plus 2 equivalents of Al^{3+} and no SiO_4^{4-}; spectrum c, 4 equivalents of Al^{3+} and no SiO_4^{4-}; spectrum d, 4 equivalents of Al^{3+} and 2 equivalents of SiO_4^{4-}; spectrum e, 4 equivalents of Al^{3+} and 4 equivalents of SiO_4^{4-}.

making of the βA4 solution or addition of the titrant and the data acquisition, to allow the peptide to reach conformational equilibrium. The initial spectrum, βA4 in trifluoroethanol without Al^{3+} or SiO_4^{4-}, is spectrum a in Fig. 1. This curve demonstrates a partial α-helical, partial random coil structure (34). Upon addition of 2 molar equivalents of Al^{3+}, the mean residue ellipiticity at 220 nm ($[\theta]_{220}$) increased, indicating the formation of β-sheets (spectrum b). On addition of another 2 equivalents of Al^{3+}, the curve (spectrum c) remained identical to the previous one. Similar conformational transitions have been observed previously (31). Immediately after completion of spectrum c, 2 equivalents of SiO_4^{4-} was titrated into βA4/Al^{3+} solution, resulting in spectrum d of Fig. 1 and causing no detectable change in structure. However, addition of 4 equivalents (total) of SiO_4^{4-} produced spectrum e. Thus a reversal of the effect of Al^{3+} was brought about by the addition of SiO_4^{4-}. The βA4 protein, which can be transformed in β-sheets by the addition of Al^{3+}, can be transformed into its initial conformation by the addition of SiO_4^{4-}.

The implications are immediately evident and suggest the possible use of silicates as a therapeutic agent for AD, since both model tangles (20) and precipitated β-pleated sheets of βA4 can be reversed to soluble forms by silicates. SiO_4^{4-} has been shown to be biocompatible, and thus no adverse side effects can be foreseen (33). To be effective in AD, a therapeutic agent needs to cross the blood–brain barrier. However, aluminum silicates have been found (8) in AD lesions in the brain. Thus, it is evident that both species Al^{3+} and SiO_4^{4-} do cross the blood–brain barrier. Silicates could be effective in reducing the Al^{3+} concentration in circulating blood, and thus aluminum would be less available to cross the blood–brain barrier.

G.D.F. is a Rosenfield Professor of Chemistry. This research was supported by a grant from the National Institute on Aging.

1. Cordell, B. (1994) *Annu. Rev. Pharmacol. Toxicol.* **34,** 69–89.
2. Scherer, R. S. (1994) *Bio/Technology* **12,** 140–144.
3. Perry, G. & Smith, M. A. (1993) *Clin. Neurosci.* **1,** 199–203.
4. Sisodia, S. S. & Price, D. L. (1993) *Clin. Neurosci.* **1,** 176–183.
5. Goddert, M. (1993) *Trends Neurosci.* **16,** 460–465.
6. Perl, D. P. (1985) *Environ. Health Perspect.* **63,** 149–153.
7. Bertholf, R. L. (1987) *CRC Crit. Rev. Clin. Lab. Sci.* **2,** 195–210.
8. Candy, J. M., Oakley, A. E., Klinowski, J., Carpenter, T. A., Perry, R. H., Atack, J. R., Perry, E. K., Blessed, G., Fairbairn, A. & Edwardson, J. A. (1986) *Lancet* **i,** 354–356.
9. Landsberg, J. P., McDonald, B. & Watt, F. (1992) *Nature (London)* **360,** 65–68.
10. Chadwick, D. J. & Whelan, J., eds. (1992) *Aluminum in Biology and Medicine,* Ciba Foundation Symposium 19 (Wiley, Chichester, U.K.).
11. Alfrey, A. C. (1991) in *Aluminum in Chemistry, Biology and Medicine*, eds. Nicolini, M., Zatta, P. F. & Corain, B. (Raven, New York), pp. 73–84.
12. Zatta, P. F. (1993) *Trace Elem. Med.* **10,** 120–128.
13. Martin, R. B. (1986) *Clin. Chem.* **32,** 1797–1806.
14. Birchall, J. D., Exley, C., Chappell, J. S. & Phillips, M. J. (1989) *Nature (London)* **338,** 146–148.
15. Epstein, E. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11–17.
16. Edwardson, J. A., Moore, P. B., Ferrier, I. N., Lilleys, J. S., Newton, G. W. A., Berker, J., Templar, J. & Day, J. P. (1993) *Lancet* **342,** 211–212.
17. Carlisle, E. M. & Curran, M. J. (1987) *Alzheimer Dis. Relat. Disord.* **1,** 83–89.
18. Hollosi, M., Urge, L., Perczel, A., Kajtar, J., Teplan, I., Otvos, L., Jr., & Fasman, G. D. (1992) *J. Mol. Biol.* **223,** 673–682.
19. Hollosi, M., Otvos L., Jr., Urge, L., Kajtar, J., Perczel, A., Laczko, I., Vadasz, Zs. & Fasman, G. D. (1993) *Bipolymers* **33,** 497–510.

20. Fasman, G. D. & Moore, C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11232–11235.
21. Perry, G. & Smith, M. A. (1993) *Clin. Neurosci.* **1,** 199–203.
22. Jarrett, J. & Landsbury, P. T., Jr. (1993) *Cell* **73,** 1055–1058.
23. Selkoe, D. J. (1994) *Annu. Rev. Neurosci.* **17,** 489–517.
24. Hilbich, C., Kisters-Woike, B., Reed, J., Masters, C. L. & Beyreuther, K. J. (1991) *J. Mol. Biol.* **218,** 149–163.
25. Barrow, C. J. & Zagorski, M. G. (1991) *Science* **253,** 179–182.
26. Barrow, C. J., Yasuda, A., Kenny, P. T. M. & Zagorski, M. G. (1992) *J. Mol. Biol.* **225,** 1075–1093.
27. Simmons, L. K., May, P. C., Tomaselli, K. J., Rydel, R. E., Fuson, K. S., Brigham, E. F., Wright, S., Lieberburg, I., Becker, G. W., Brems, D. N. & Li, W. Y. (1994) *Mol. Pharmacol.* **45,** 373–379.
28. Tomski, S. & Murphy, R. M. (1992) *Arch. Biochem Biophys.* **294,** 630–638.
29. Terzi, G. E., Hölzemann, G. & Seelig, J. (1994) *Biochemistry* **33,** 1345–1350.
30. Halverson, K., Frasier, P. E., Kirschner, D. A. & Landsbury, P. T., Jr. (1990) *Biochemistry* **29,** 2693–2644.
31. Exley, C., Price, N. C., Kelley, S. M. & Birchall, J. D. (1993) *FEBS Lett.* **324,** 293–295.
32. Kawahara, M., Maramoto, K., Kobayashi, K., Mori, H. & Kuroda, Y. (1994) *Biochem. Biophys. Res. Commun.* **198,** 531–535.
33. Hench, L. L. & Wilson, J. (1986) in *Silicon Biochemistry*, Ciba Foundation 121, eds. Everest, D. & O'Conner, M. (Wiley, Chichester, U.K.), pp. 231–246.
34. Greenfield, N. J. & Fasman, G. D. (1969) *Biochemistry* **8,** 4108–4116.
35. Fields, G. B. & Noble, R. L. (1990) *Int. J. Pept. Protein Res.* **35,** 161–214.