Amyloid β-Peptide Possesses a Transforming Growth Factor-β Activity*

(Received for publication, July 23, 1998)

Shuan Shian Huang‡, Franklin W. Huang‡§, Jan Xu‡, Shawei Cheng‡, Chung Y. Hsu‡, and Jung San Huang‡¶

From the ‡Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, Missouri 63104 and ¶Center for the Study of Nervous System Injury and Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

Amyloid β-peptide (Aβ) of 39–42 amino acid residues is a major constituent of Alzheimer’s disease neurite plaques. Aβ aggregates (fibrils) are believed to be responsible for neuronal damage and dysfunction, as well as microglia and astrocyte activation in disease lesions by multiple mechanisms. Since Aβ aggregates possess the multiple valencies of an FAED motif (20th to 23rd amino acid residues), which resembles the putative transforming growth factor-β (TGF-β) active site motif, we hypothesize that Aβ monomers and Aβ aggregates may function as TGF-β antagonists and partial agonists, analogous to previously described monovalent and multivalent TGF-β peptide antagonists and agonists (Huang, S. S., Liu, Q., Johnson, F. E., Konish, Y., and Huang, J. S. (1997) J. Biol. Chem. 272, 27155–27159). Here, we report that the Aβ monomer, Aβ-(1–40) and its fragment, containing the motif inhibit radiolabeled TGF-β binding to cell-surface TGF-β receptors in mink lung epithelial cells (Mv1Lu cells). Aβ-(1–40)-bovine serum albumin conjugate (Aβ-(1–40)-BSA), a multivalent synthetic analogue of Aβ aggregates, exhibited cytotoxicity toward bovine cerebral endothelial cells and rat postmitotic differentiated hippocampal neuronal cells (H19-7 cells) and inhibitory activities of radiolabeled TGF-β binding to TGF-β receptors and TGF-β-induced plasminogen activator inhibitor-1 expression, that were ~100–670 times more potent than those of Aβ-(1–40) monomers. At less than micromolar concentrations, Aβ-(1–40)-BSA but not Aβ-(1–40) monomers inhibited proliferation of Mv1Lu cells. Since TGF-β is an organizer of responses to neurodegeneration and is also found in neurite plaques, the TGF-β antagonist and partial agonist activities of Aβ monomers and aggregates may play an important role in the pathogenesis of the disease.

Amyloid β-peptide (Aβ)† of 39–42 amino acid residues comprises the major protease-sensitive component of amyloid deposits in the brains of patients with Alzheimer’s disease (1–6). The deposition of Aβ aggregates (fibrils) is believed to be an early and critical event in the pathogenesis of Alzheimer’s disease. The mechanisms by which Aβ aggregates exert their detrimental effects are not well understood, but may involve effects through interactions with specific cell-surface receptors or binding proteins. Several receptors and binding proteins have been reported to interact with Aβ, but none appears to be able to discriminate Aβ monomers from Aβ aggregates (7–9).

Recently, we have identified a putative TGF-β active-site motif (WSXD) in TGF-β isomers (TGF-β1 and TGF-β2) (10). Synthetic peptides containing this motif in the middle of the peptide exhibit TGF-β antagonist activity. Multiple conjugation of these peptides to carrier proteins not only enhances TGF-β antagonist activity but also confers partial TGF-β agonist activity (10). Since Aβ contains a motif (FAED) that is similar to the putative TGF-β active-site motif (WSXD) and since Aβ aggregates would provide multivalencies with many copies of the putative active-site motif (11), we hypothesize that the Aβ monomer and Aβ aggregates may function as TGF-β antagonist and partial TGF-β agonist, analogous to previously described monovalent and multivalent TGF-β peptide antagonist/partial agonist, respectively (10). To test this hypothesis, we investigated the TGF-β antagonist/agonist activity of the Aβ-(1–40) monomer and Aβ-(1–40)-bovine serum albumin conjugate (Aβ-(1–40)-BSA) which contains ~5–10 Aβ-(1–40) peptides per molecule of protein and mimics Aβ aggregates in multivalencies (11). In this communication, we demonstrate that Aβ-(1–40) monomers inhibited 125I-labeled TGF-β binding to cell-surface TGF-β receptors in mink lung epithelial cells (Mv1Lu cells). We also show that Aβ-(1–40)-BSA exhibited a potent cytotoxicity toward bovine cerebral endothelial (BCE) cells and rat post-mitotic differentiated hippocampal neuronal cells (H19-7 cells), and strongly inhibited 125I-TGF-β binding to cell-surface TGF-β receptors and TGF-β-induced expression of plasminogen activator inhibitor 1 (PAI-1). Aβ-(1–40)-BSA but not Aβ-(1–40) monomers inhibited cellular proliferation of Mv1Lu cells.

EXPERIMENTAL PROCEDURES

Materials—Na125I (17 Ci/mmol) and [methyl-3H]Thymidine (67 Ci/mmol) were purchased from ICN Radiochemicals (Irvine, CA). High molecular mass protein standards (myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase, 97 kDa; bovine serum albumin, 66 kDa), Aβ-(1–16), Aβ-(1–20), and Aβ-(1–42) were obtained from Sigma. Aβ-(1–40), Aβ-(25–35), and Aβ-(12–28) were obtained from Bachem Bioscience, Inc. (King of Prussia, PA). Disuccinimidyl suberate was obtained from Pierce. TGF-β1 was purchased from Austral Biologicals (San Ramon, CA). β2(41–45), a synthetic pentacosapeptide with an amino acid sequence corresponding to the 41st to 65th amino acid residues of TGF-β1, was synthesized as described previously (10). Mv1Lu cells were grown in 10% fetal calf serum in Dulbecco’s modified Eagle’s medium.

Preparation of BSA Conjugates of Aβ-(1–40) and Aβ Fragments—

* This work was supported by the National Institutes of Health Grant CA38808. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Summer research student from Harvard University.

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, MO 63104. Tel: 314-577-8135; Fax: 314-577-8156; E-mail: huangjs@wpogate.slu.edu.

The abbreviations used are: Aβ, amyloid β-peptide; TGF, transforming growth factor; BSA, bovine serum albumin; BCE, bovine cerebral endothelial; PAI, plasminogen activator inhibitor; FAD, familial Alzheimer’s disease; H19-7, rat post-mitotic differentiated hippocampal neuronal cells; TGF-β, TGF-β receptor; Mv1Lu, mink lung epithelial.
Amino acid sequences of TGF-β peptide antagonists (β25-(41–65) and β25-(41–65) Aβ-(1–40), and Aβ fragments. The amino acid residues underlined are the putative TGF-β active-site motifs. Identical amino acid residues are boxed with a solid line, whereas functionally homologous residues are boxed with a broken line. Dutch, Dutch-type Alzheimer's disease.

RESULTS AND DISCUSSION

Aβ and its fragments have been found to exert cytotoxic and trophic effects on cells in culture. Aβ(25–35) mimics the cytotoxic activity of Aβ (5), whereas Aβ(1–28), Aβ(25–28), and Aβ(1–40) exhibit acetylcholine release inhibitory activity (4). The sequence of VFF (residues 18–20) has been implicated in mediating the amnestic activity of Aβ fragments (17). These cytotoxic and trophic effects of Aβ appear to be mediated by different domains, e.g., the sequences of residues 25–35, 25–28, and 18–20 (4, 5, 17). We noted that, in addition to these domains, Aβ possesses a motif (FAEĐ) 20th to 23rd amino acid residues), which is similar to the WSXWSX putative TGF-β active-site motif. Known TGF-β peptide antagonists β25-(41–65) and β25-(41–65) that contain this motif are synthetic peptides with amino acid sequences corresponding to the 41st to 65th residues of TGF-β1 and TGF-β2, respectively (Fig. 1) (10). Replacement of the tryptophan residue in the motif by a phenylalanine residue does not affect the antagonist activity of β25-(41–65). Thus, the Aβ monomer or a functional TGF-β active-site motif. To test this possibility, we determined the effects of Aβ(1–40) monomers and Aβ fragments possessing and lacking the FAED motif on 125I-TGF-β1 binding to cell-surface TGF-β receptors in MvLu cells, a standard model system for investigating TGF-β receptor types and TGF-β-induced cellular responses (12, 13). As shown in Fig. 2, Aβ(1–40), and Aβ(12–28), both of which contain the motif, exhibited 125I-TGF-β1 receptor binding inhibitory activities with IC50 of ~3 and ~30 μM, respectively. Aβ(25–35), Aβ(1–20), and Aβ(1–16), all of which lack the motif, failed to show 125I-TGF-β1 binding inhibitory activity at any concentration up to 30 μM. These results indicate that Aβ(1–40) possesses a functional TGF-β active-site motif.

It has been reported that the aggregation of Aβ monomers is not easily controlled in vitro, as it is strongly affected by Aβ concentration, pH, ionic strength, and incubation time (18–22). In order to produce a multivalent stable inhibitor, we prepared Aβ(1–40)-bovine serum albumin conjugate (Aβ(1–40)-BSA, Mw ~90,000–100,000) containing ~5–10 Aβ(1–40) per molecule of BSA according to the procedure of Huang, et al. (10). Unlike the rather unstable Aβ(1–40) aggregates, Aβ(1–40)-BSA is stable (at 4°C) for at least a few weeks and has a consistent valence due to the covalent nature of the attachment of Aβ(1–40) to BSA. This conjugate is meant to mimic Aβ(1–40) aggregates by possessing multiple valences of Aβ(1–40) per molecule and cytotoxicity toward BCE cells and rat postmitotic differentiated H19-7 cells. As shown in Fig. 3, Aβ(1–40)-BSA exhibited cytotoxicity toward BCE cells and H19-7 cells that was ~670 times more potent than that of the Aβ(1–40) monomer (Fig. 3, A and B). Aβ(1–40)-BSA at 75 μM was as potent as 50 μM Aβ(1–40) in causing cell death of BCE cells and H19-7 cells as determined by the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (16) and trypan blue exclusion assay (14). Similar cytotoxic effects of Aβ(1–40)
and Aβ-(1–40)-BSA in BCE cells and H19-7 cells were also noted using lactate dehydrogenase assay (14) and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, respectively (data not shown).

To determine the TGF-β antagonist activity of Aβ-(1–40)-BSA, we examined the 125I-TGF-β1 receptor binding inhibitory activity of Aβ-(1–40)-BSA in BCE cells and H19-7 cells, and Aβ-(1–40) and Aβ-(1–40)-BSA in BCE cells and H19-7 cells.

Fig. 2. Effects of Aβ-(1–40) and Aβ fragments on 125I-TGF-β1 binding in Mv1Lu cells. Cells were incubated with 0.1 nM 125I-TGF-β1 and various concentrations of Aβ-(1–40), Aβ-(12–28), Aβ-(25–35), Aβ-(1–20), and Aβ-(1–16) at 0 °C for 2.5 h. The specific binding of 125I-TGF-β1 without Aβ-(1–40) and Aβ fragments was taken as 0% inhibition (5,429 ± 780 cpm/well). The error bars are means ± S.D. of triplicate cell cultures. The data are representative of seven experiments that gave comparable results.

Fig. 3. Cytotoxic effects of Aβ-(1–40) and Aβ-(1–40)-BSA in BCE cells and H19-7 cells. A, BCE cells were exposed to Aβ-(1–40) or Aβ-(1–40)-BSA at concentrations indicated for 48 h. The extent of cell survival (mean ± S.D.) was determined in quadruplicate cell cultures by the MTT assay. The asterisk denotes a p value of <0.05. The data are representative of two experiments that gave comparable results.

B, H19-7 cells were exposed to Aβ-(1–40) or Aβ-(1–40)-BSA at concentrations indicated for 24 h. The extent of cell survival (mean ± S.D.) was determined in quadruplicate cell cultures by the trypan blue exclusion assay. The asterisk denotes a p value of <0.05. The data are representative of two experiments that gave comparable results.

Fig. 4. Effects of the BSA conjugates of Aβ-(1–40) and its fragments on 125I-TGF-β1 binding (A) and 125I-TGF-β1-affinity labeling (B) in Mv1Lu cells. A, cells were incubated with various concentrations of the BSA conjugates of Aβ-(1–40), Aβ-(12–28), Aβ-(25–35), or Aβ-(1–16) at 0 °C for 25 h. The specific binding of 125I-TGF-β1 without the BSA conjugates was taken as 0% inhibition. The error bars are means ± S.D. of triplicate cell cultures. The data are representative of five experiments that gave comparable results. B, after 125I-TGF-β1 binding, in the absence (control) and presence of 10 μM β1(41–65), 0.1 μM Aβ-(1–40)-BSA, or 5 μM Aβ-(1–40), the 125I-TGF-β1 affinity labeling of cell-surface TGF-β receptors was performed. The 125I-TGF-β1 affinity-labeled receptors were analyzed by 5% SDS-polyacrylamide gel electrophoresis and autoradiography. The brackets indicate the locations of the types I, II, III, and V TGF-β receptors (TβR-I, TβR-II, TβR-III, and TβR-V).
scripts on the autoradiograms were estimated by a PhosphorImager. Neither Aβ-(1–40) monoclonal antibodies nor DNA synthesis was determined by measuring [methyl-3H]thymidine incorporation into cellular DNA. The assay was carried out in triplicate cell cultures.

5 μM (Fig. 4B). These results indicate that the multiple valencies enhance the 125I-TGF-β1-binding-inhibitory activity of Aβ-(1–40).

Dimerization is known to be required for TGF-β activity (23) and multivalent TGF-β peptide antagonists have been shown to exhibit partial TGF-β agonist activity as assayed by growth inhibition (10). We therefore examined the TGF-β agonist activity of Aβ-(1–40)-BSA by measuring its inhibition of DNA synthesis using Mv1Lu cells. As shown in Fig. 5A, 0.35 μM of multivalent Aβ-(1–40)-BSA produced 35% inhibition of [methyl-3H]thymidine incorporation into DNA of Mv1Lu cells. Neither Aβ-(1–40) monomers (0.35 μM) nor BSA conjugated in the absence of peptide (at any concentration up to 10 μM) affected DNA synthesis in this system. The DNA synthesis inhibition induced by 0.35 μM Aβ-(1–40)-BSA was blocked in the presence of 10 μM Aβ-(25–35), a specific TGF-β receptor antagonist (data not shown). These results suggest that multiple valencies of Aβ-(1–40) confer TGF-β agonist activity, i.e., inhibit cellular proliferation as measured by DNA synthesis. To support this suggestion, we determined the effect of Aβ-(1–40)-BSA on DNA synthesis of type I TGF-β receptor-defective mutant and wild-type mink lung epithelial cells (R1B and Mv1Lu cells) (12, 24). If the DNA synthesis inhibition by Aβ-(1–40)-BSA is mediated by cell surface TGF-β receptors, R1B cells, which lack expression of the functional type I TGF-β receptor (12, 24), should respond very little if any to Aβ-(1–40)-BSA DNA synthesis inhibition. As shown in Table I, Aβ-(1–40)-BSA did not significantly affect DNA synthesis of R1B cells. This result is consistent with the suggestion that the Aβ-(1–40)-BSA exhibits TGF-β agonist activity in growth inhibition.

The transcriptional expression of PAI-1 is known to be stimulated by TGF-β1 (25–27). To determine whether Aβ-(1–40)-BSA interferes with this effect, Mv1Lu cells were treated with 0.5 μM TGF-β1 plus various concentrations of Aβ-(1–40)-BSA for 2 h at 37 °C. Northern blot analysis was then performed. As shown in Fig. 5B, Aβ-(1–40)-BSA did not stimulate the expression of PAI-1 (lane 2 versus lane 1). However, Aβ-(1–40)-BSA diminished the PAI-1 expression stimulated by 0.5 μM TGF-β1 in a dose-dependent manner (lanes 4–6). This suggests that Aβ-(1–40)-BSA can bind to TGF-β receptors and function as an antagonist for TGF-β as assayed by transcriptional activation.

In summary, Aβ-(1–40)-BSA is a stable multivalent analogue of naturally occurring Aβ aggregates seen in Alzheimer's disease lesions and is more potent than Aβ-(1–40) as a TGF-β antagonist that blocks TGF-β binding to TGF-β receptors. The cytotoxicity of Aβ-(1–40)-BSA toward BCE cells and H19-7 cells is ~670 times more potent than that of Aβ-(1–40). Furthermore, Aβ-(1–40)-BSA, which has multiple Aβ-(1–40) peptides per BSA molecule, possesses partial TGF-β agonist activity (growth inhibition). These results suggest that Aβ monomers and Aβ aggregates may participate in the pathogenesis of neuronal death in Alzheimer's disease patients through their TGF-β antagonist and agonist activities. TGF-β has been shown to protect neurons from cell death (28–32). Since TGF-β expression has been detected in Alzheimer's disease lesions (28, 33–35), we hypothesize that the TGF-β antagonist activity (TGF-β receptor binding inhibitory activity) of Aβ-(1–40) monomers and aggregates may counteract this neuroprotective effect of TGF-β. As both glial cells and monocytes have been shown to express TGF-β (35) and to respond to TGF-β stimulation (28), the partial TGF-β agonist activity (growth inhibition) of Aβ aggregates may also play an important role in the chemotaxis and activation of astrocytes and microglia that are associated with Alzheimer's disease.

The familial Alzheimer's disease (FAD) (36) and Dutch-type Alzheimer's disease (37) patients may provide some clues to the structure/function relationship of the putative TGF-β active-site motif (FAED) in Aβ, since these patients have mutations within this motif (Fig. 1). The mutations in both FAD (A692G) and Dutch-type (E693Q) patients may provide a TGF-β active-site motif with particularly robust function on the basis of studies of various motifs in synthetic TGF-β peptide antagonists (10). If the 2nd and 3rd amino acid residues in the motif are amino acids with small side chains (Gly, Ser, Cys, and Ala

| Table I | Effect of Aβ-(1–40)-BSA and Aβ-(1–40) on DNA synthesis of type I TGF-β receptor-defective and wild-type mink lung epithelial cells (R1B and Mv1Lu cells) |
|--------|-------------------------------------------------|
| R1B cells | Mv1Lu cells |
| [methyl-3H]Thymidine incorporation | cpm/well |
| Control | 4890 ± 240 | 23,940 ± 1,520 |
| +0.35 μM Aβ-(1–40)-BSA | 4520 ± 480 | 15,082 ± 929 |
| +0.5 μM Aβ-(1–40) | 5019 ± 320 | 24,120 ± 2,001 |

* Cells were incubated with 0.35 μM Aβ-(1–40)-BSA or 10 μM Aβ-(1–40) at 37 °C for 16 h. DNA synthesis was determined by measuring [methyl-3H]thymidine incorporation into cellular DNA. The assay was carried out in triplicate cell cultures.

FIG. 5. Effects of BSA conjugates of Aβ-(1–40) and Aβ fragments on DNA synthesis (A) and TGF-β1-induced PAI-1 expression (B) in Mv1Lu cells. A, cells were incubated with various concentrations of Aβ-(1–40) and BSA conjugates of Aβ-(1–40), Aβ-(12–28), Aβ-(25–35), and Aβ-(1–20) at 37 °C for 16 h. DNA synthesis was determined by measuring [methyl-3H]thymidine incorporation into cellular DNA. The [methyl-3H]thymidine incorporations in the presence and absence of 10 μM TGF-β1 were taken as 100 and 0% inhibition (2,242 ± 679 and 25,493 ± 1,200 cpm/well, respectively). The error bars are means ± S.D. of triplicate cell cultures. The data are representative of six experiments which gave comparable results. B, cells were treated with 0.5 μM TGF-β1, in the presence of various concentrations of Aβ-(1–40)-BSA at 37 °C for 2 h. PAI-1 expression was determined by Northern blot analysis. The expression of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as a control. The relative intensities of transcripts on the autoradiograms were estimated by a PhosphorImager.
residues) and noncharged amino acids, respectively, in the TGF-β peptide antagonist motif (XXD), the potency of TGF-β antagonism is enhanced. Determining the TGF-β activities of FAD and Dutch-type β mutant peptides would test the hypothesis that the TGF-β activities of these peptides are important in the mechanism of Aβ in the neuronal degeneration of Alzheimer’s disease.

Acknowledgments—We thank Drs. William S. Sly, Frank E. Johnson, and Uthay Ezekiel for critical comments and review of the manuscript and John McAlpin for preparing the manuscript.

REFERENCES
1. Selkoe, D. J. (1997) J. Neuropathol. Exp. Neurol. 56, 438–447
2. Lendon, C. L., Ashall, E., and Goate, A. M. (1997) J. Am. Med. Assoc. 277, 825–831
3. Hardy, J. (1997) Trends Neurosci. 20, 154–159
4. Auld, D. S., Kar, S., and Quirion, R. (1998) Trends Neurosci. 21, 43–48
5. Yankner, B., Duffy, L., and Kirschner, D. (1990) J. Neurochem. 53, 154–159
6. Pike, C. J., Walencewicz, A., Glabe, C., and Cotman, C. W. (1993) J. Cell. Biochem. 53, 689–695
7. Yaar, M., Zhai, S., Pilch, P. F., Doyle, S. M., Eisenhauer, P. B., Fine, R. E., and Gilchrest, B. A. (1997) J. Clin. Invest. 99, 2333–2340
8. Prehn, J. H. M., Bindokas, V. P., Marcucilli, C. J., Krajewski, S., Reed, J. C., and Miller, R. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12599–12603
9. Prehn, J. H. M., Bindokas, V. P., Jordan, J., Galindo, M. F., Ghadge, G. D., Roos, R. P., Buise, J. H. Thompson, C. B., Krajewski, S., Reed, J. C., and Miller, R. (1996) Mol. Pharmacol. 49, 319–328
10. van der Wal, E. A., Côme-Pinilla, E., and Cotman, C. W. (1993) NeuroReport 4, 69–72
11. Prese, N. S., and Perillo, E. (1995) J. Neuropathol. Exp. Neurol. 54, 802–811
12. Hendriks, L., van Duijn, C. M., Cras, P., Crus, M., Hul, W. U., van Harkamp, F., Warren, A., McInnis, M. G., Antonarakis, S. I., Martin, J.-J., Hofman, A., and Broekhoven, C. (1992) Nat. Genet. 1, 218–221
13. Levy, E., Carman, M. D., Fernandez-Madrid, L. J., Power, M. D., Lieberburg, I., van Duinen, S. G., Bots, G. T. A. M., Luyendijk, W., and Frangione, B. (1990) Science 248, 1124–1126