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RESEARCH PAPER

# Reversal of temperature-induced conformational changes in the amyloid-beta peptide, Aβ40, by the β-sheet breaker peptides 16–23 and 17–24

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**Background and purpose:** Aggregates of the protein amyloid-beta (Aβ) play a crucial role in the pathogenesis of Alzheimer's disease (AD). Most therapeutic approaches to AD do not target Aβ, so determination of the factor(s) that facilitate aggregation and discovering agents that prevent aggregation have great potential therapeutic value.
**Experimental approach:** We investigated *ex vivo* the temperature-sensitive regions of Aβ1–40 (Aβ40) and their interactions with octapeptides derived from sequences within Aβ40 – β-sheet breaker peptides (βSBP) – using enzyme-linked immunosorbent assay, and dot blot and far-UV circular dichroism (CD) spectroscopy. We measured changes within the physiological limits of temperature, using antibodies targeting epitopes 1–7, 5–10, 9–14 and 17–21 within Aβ40.
**Key results:** Temperature-dependent conformational changes were observed in Aβ40 at epitopes 9–14 and 17–21 at 36–38 and 36–40°C respectively. The βSBPs 16–23 and 17–24, but not 15–22 and 18–25, could inhibit the changes. Moreover, βSBPs 16–23 and 17–24 increased digestion of Aβ40 by protease K, indicating a decreased aggregation of Aβ40, whereas βSBPs 15–22 and 18–25 did not increase this digestion. CD spectra revealed that β-sheet formation in Aβ40 at 38°C was reduced with βSBPs 16–23 and 17–24.
**Conclusions and implications:** The epitopes 9–14 and 17–21 are the temperature-sensitive regions within Aβ40. The βSBPs, Aβ16–23 and 17–24 reversed temperature-induced β-sheet formation, and decreased Aβ40 aggregation. The results suggest that the 17–23 epitope of Aβ40 is crucially involved in preventing Aβ40 aggregation and consequent deposition of Aβ40 in AD brain.
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**Keywords:** amyloid β1–40; temperature; β-sheet formation; breaker peptides; Alzheimer's disease treatment

**Abbreviations:** Aβ, amyloid beta; AD, Alzheimer's disease; βSBP, beta-sheet breaker peptide; CD, circular dichroism; PK, protease K

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive impairment. The hallmark of AD pathogenesis in brain is amyloid plaques composed mainly of amyloid-beta (Aβ) protein aggregates (Katzman and Saitoh, 1991). It is believed that β-sheet formation is the general mechanism of aberrant protein aggregation leading to AD (Walsh *et al.*, 1999). Specific sequences within the main Aβ structure are involved in the structural transformation and the toxic effects of Aβ (Simmons *et al.*, 1994). The hydrophobic core around residues 17–20 of Aβ1–40 (Aβ40) (Lui *et al.*, 2004), and protein misfolding process in which intermolecular β-sheet interactions become stabilized abnormally (Huang *et al.*, 2000; McAllister *et al.*, 2005) are crucial for the formation of the β-sheet structure. Moreover, C-terminal fragments are more harmful than N-terminal fragments of Aβ, and may induce the development of dystrophic neurites by a toxic effect rather than by physical injury (Lin *et al.*, 2001; Kasa *et al.*, 2003). Recent reports suggested that soluble Aβ oligomers extracted directly from AD brain potentially impair synaptic structure and function, and that the Aβ N-terminus is the key sequence causing the cognitive impairment; however, insoluble Aβ did not impair the synaptic function (Cleary *et al.*, 2005; Shankar *et al.*, 2008).

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The structural form of Aβ is influenced by a variety of intrinsic, as well as extrinsic, factors that cause conformational transition of Aβ from a random-coil to the predominantly β-sheet structure. These factors include peptide concentration (Barrow *et al.*, 1992), low pH (Matsunaga *et al.*, 2002; Petkova *et al.*, 2004), metal ions (Drago *et al.*, 2007), high cholesterol (Kakio *et al.*, 2001; Yanagisawa and Matsuzaki, 2002) and pressure (Lin *et al.*, 2002). Moreover, temperature-dependent transition of Aβ40 plays an important role in the structural transformation from α-helix and random-coil to β-sheet form in aqueous solution by heating above 37°C (Gursky and Aleshkov, 2000) or at 45°C (Lin *et al.*, 2003).

The available drugs used in the treatment of AD mainly aim at increasing the cholinergic activity of the remaining healthy neurons, but do not act on the main cause of the disease. One of the therapeutic approaches was vaccination against the N- and C-terminals of Aβ. Passive immunization against the C-terminal increased brain-soluble Aβ42/43, decreased insoluble Aβ40 and Aβ42/43 and reduced plaque formation (Asami-Odaka *et al.*, 2005). However, the appearance of severe side effects during clinical trials has highlighted the need for improved safety and efficacy. In addition, low levels of anti-Aβ antibodies can be detected in individuals with or without AD, and their presence or levels are not correlated with the likelihood of developing dementia (Hyman *et al.*, 2001).

Accordingly, safer compounds preventing and reversing cerebral deposition of Aβ, and thus lowering the burden of insoluble Aβ have become an attractive therapeutic strategy for AD. It has been found that Aβ aggregation can be selectively inhibited with short synthetic peptides designed as β-sheet breaker peptides (βSBPs) (Synder *et al.*, 1994). *In vitro* cell culture and *in vivo* results suggest that βSBPs might be candidates for AD therapy directed towards reducing amyloid deposition (Permanne *et al.*, 2002a). Two pentapeptide βSBPs have been synthesized. One contains the same sequence as residues 16–20 (KLVFF) within Aβ (Tjernberg *et al.*, 1996), and the other is a five-residue synthetic peptide (iAβ5: LPFFD) homologous to the central hydrophobic fragment of Aβ 17–21 (LVFFA) with substitution of P for V, and D for A (Soto *et al.*, 1996). Both βSBP 16–20 and 17–21 could inhibit Aβ fibrillogenesis (Hetenyi *et al.*, 2002). Further, the βSBP 17–21 could prevent β-sheet formation, inhibit and disassemble amyloid fibrils *in vitro* and also prevent Aβ neurotoxicity in cell culture (Soto *et al.*, 1998) by stabilizing the normal conformation and destabilizing the β-sheet-rich structure (Soto *et al.*, 2000), reversing pre-existing Aβ fibrils (Sigurdsson *et al.*, 2000) or preventing formation of the amyloid plaques (Permanne *et al.*, 2002a). An end-protected version of iAβ5, acetylated at the N-terminus and amidated at the C-terminus (iAβ5p) with high rate of penetration across the blood–brain barrier had been synthesized. It has been reported that iAβ5 is able to induce a dramatic reduction in amyloid deposition and the associated brain inflammation, and increase neuronal survival (Permanne *et al.*, 2002b).

The present study aimed at detecting temperature-sensitive regions within Aβ40, and determining whether or not temperature-induced changes are inhibited or reversed by octapeptide βSBPs (corresponding to residues 15–22, 16–23, 17–24 and 18–25 in Aβ40) using enzyme-linked immunosorbent assay (ELISA), Western dot blots and far-UV circular dichroism (CD) spectra analysis.

## Methods

*Temperature modification of Aβ peptides and ELISA assay*

ELISA was conducted for both Aβ40 alone and its mixture with βSBPs. Samples of Aβ40 alone (10 $\mu g \cdot mL^{-1}$) were incubated from 35 to 42°C with 1°C intervals, and also incubated at 20°C as a control for soluble Aβ40, for 30 min in tubes, then 50 μL of each solution was bound to the wells of the flat bottom high polystyrene microtitre plates overnight at the same temperature at which it had been incubated. In a similar way, Aβ40 (10 $\mu g \cdot mL^{-1}$) was also incubated after mixing with 10 $\mu g \cdot mL^{-1}$ of each βSBP: Aβ15–22, 16–23, 17–21 and 18–25. After the removal of excess samples, the wells were first incubated for 2 h with Tris-buffered saline (TBS; 20 mM Tris/34 mM NaCl, pH 7.4) containing 3% bovine serum albumin (BSA) at 37°C. After discarding the solution, primary antibody (50 μL of 1 $\mu g \cdot mL^{-1}$ of either 4G8, 6F/3D, anti 5–10 or anti 1–7) in TBS containing 1% BSA was incubated for a further 2 h at 37°C, pH 7.4. After incubation, the wells were washed with TBS with 0.1% Tween-20, pH 7.4 (TBST), and incubated for an additional 1 h at 37°C with 50 μL of a 1:5000 dilution of alkaline phosphatase-conjugated secondary antibody. After washing with TBST, bound antibodies were detected by the addition of *p*-nitrophenyl phosphate, and the absorbance was measured after 30 min at 405 nm using a spectrophotometric plate reader (Microplate reader MPR A4I, Tosoh, Tokyo, Japan). All washing steps were performed six times. The same procedure was applied for Aβ40 and βSBP mixtures.

*Dot blot*

A preliminary investigation was conducted using βSBPs at 1, 5, 10, 15 and 20 $\mu g \cdot mL^{-1}$ concentrations, and showed that βSBP at 10–20 $\mu g \cdot mL^{-1}$ produced dark spots, indicating that high amounts of protein remain on the membrane and the spots were completely digested with protease K (PK) at 0.05 $mg \cdot mL^{-1}$. Accordingly, we carried out dot blot studies on Aβ40 at 8 $\mu g \cdot mL^{-1}$ with or without βSBPs at 20 $\mu g \cdot mL^{-1}$, which corresponds to a molar ratio of 1:12, Aβ40 : βSBP. Temperature-modified mixtures of Aβ40 and βBSP (200 μL) or Aβ40 alone at 20°C as a control for soluble Aβ40 were spotted and blotted onto methanol-immersed PVDF membrane (0.2 μm pore size; Invitrogen, Carlsbad, CA, USA) using the dot-blot apparatus (DP-48 Dot Plate; Advantec, Tokyo, Japan) by absorption with a vacuum pump. The membrane was then removed, rinsed in phosphate-buffered saline (PBS) and digested with PK.

*PK digestion and dot blots*

Each membrane was incubated without and with 10 mL of PK solution (0.05 $mg \cdot mL^{-1}$ in PBS, pH 7.4) for 1 h at 37°C with constant shaking. After removal of the PK solution, the reaction was terminated by washing with PBS with 0.1% Tween-20, pH 7.4 (PBST) three times at 15 min intervals. After blocking with 3% non-fat milk for 2 h, the membrane was

again washed with PBST and allowed to react with primary antibody 6E10 (1:10 000 dilution in PBS) for 2 h at room temperature, then the secondary antibody peroxidase-linked anti-mouse IgG (1:5000 dilution in PBS) was added and allowed to react for 1 h at room temperature. The membrane was washed three times with PBST, and the spots were detected with enhanced chemiluminescence (Immobilon, Western Chemiluminescent HRP substrate, Millipore Corp., Bedford, MA, USA) according to the manufacturer's instructions.

*CD spectroscopy analysis*

CD spectra were measured using a J-725 CD spectrometer (JASCO, Tokyo, Japan). For the far-UV CD spectra, 1 mm path length quartz cell (300 μL internal volume) was used, with bandwidth of 1 nm. Aβ40 was dissolved in 5 mM Tris-buffer (pH 9) at 1 mM, and diluted 20-fold by pure water to a final concentration of 50 μM (Bartolini *et al*., 2007). The βSBPs of Aβ15–22, 16–23, 17–24 and 18–25 were dissolved in 0.4 M NaOH at 1 mM, and adjusted with HCl to pH 7 and diluted by pure water into the same final concentration (50 μM). The mixture of Aβ40 and βSBP at 1:1 mole ratio or Aβ40 alone was incubated at 38 and 20°C as a control of soluble Aβ40 for 30 min, and spectra were recorded, using 2 nm step and a 1 s averaging time and 100 nm $min^{-1}$ scan speed.

*Statistical analyses*

For ELISA measurement, values are presented as mean ± SEM of six experiments, each in triplicate. Differences between 6F/3D antibody and other antibodies at each temperature were analysed using unpaired *t*-tests. Data of βSBPs are also presented as mean ± SEM of six experiments in triplicate, and the differences between each (βSBP and Aβ) mixture and Aβ40 alone were analysed using unpaired *t*-tests. The values of pixel densities from the dot blot studies at 38°C were expressed as mean ± SEM of six experiments, and statistical analysis was by analysis of variance.

*Materials*

*Aβ peptides and βSBPs*. Aβ40, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV were purchased from AnaSpec (San Jose, CA, USA) and dissolved in water as a stock solution, and diluted with PBS, pH 7.3, to the indicated concentrations, and used for ELISA and dot blot study. For CD study, PBS interferes with CD spectra, and we diluted the stock Aβ40 with pure water. The βSBPs: Aβ15–22, 16–23, 17–24 and 18–25 were purchased from Wako (Tokyo, Japan). All βSBPs were dissolved in a minimal amount of dimethylsulphoxide (DMSO) for ELISA and dot blot study, or dissolved in NaOH (0.4 M) for CD spectra analysis before dilution with water at the indicated concentrations.

*Monoclonal and polyclonal antibodies*. The monoclonal antibodies used were 4G8, epitope 17–21 (Signet Pathology Systems, Inc., Dedham, MA, USA); 6F/3D, epitope 9–14 (DAKO, Glostrup, Denmark); anti 5–10, epitope 5–10 (QCB, Camarillo, CA, USA); and 6E10, epitope 3–8 (COVANCE, Berkeley, CA, USA). The polyclonal anti 1–7 was from QCB. Alkaline phosphatase-conjugated goat anti-mouse IgG for monoclonal antibody and anti-rabbit IgG for polyclonal antibody (Promega, Madison, WI, USA) in ELISA, and peroxidase-labelled anti-mouse IgG (Amersham Life Science, Pharmacia Biotech, Uppsala, Sweden) for dot blots were used as secondary antibody respectively.

*Chemicals*

BSA and *p*-nitrophenyl phosphate (Sigma, St Louis, MO, USA), PK (Wako) were used. Other chemicals were from Sigma-Aldrich (Tokyo, Japan).

## Results

*Reactivity of antibodies against temperature-modified Aβ40*

In order to determine whether the temperature was significant in the ELISA after immobilization of Aβ peptides, the OD was measured as a function of temperature at 36, 37, 40 and 42°C with primary antibody for 2 h and secondary antibody for 1 h. After immobilization, the samples were unaffected by the changes to 37°C, but it also showed a corresponding slight increase of OD by 0.03–0.04 in signal if it was incubated at 42°C (data not shown). We confirmed that the conformational changes induced by overnight incubation are preserved after immobilization in the plates.

The antibodies investigated in this study displayed a different reactivity against temperature-modified Aβ40 (Figure 1). No obvious differences were detected between control samples at 20 and 35°C. A significant difference was detected among the antibodies ($P < 0.001$). Moreover, the effect of each antibody was significantly different at various temperatures ($P < 0.05$). However, the antibodies–temperature interactions did not reach significance, suggesting that the effects of

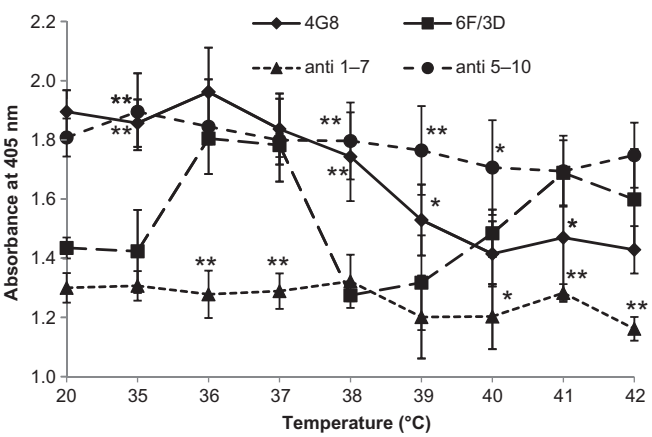


**Figure 1** Enzyme-linked immunosorbent assay (ELISA) measurements of antibody affinity towards temperature-modified Aβ40. The reactivities of four antibodies (4G8, 6F/3D, anti 1–7, anti 5–10) to samples of Aβ40 that had been exposed to temperatures over the range 36–42°C at 1°C interval and at 20°C as a control, were monitored by ELISA. Statistical comparisons were performed with unpaired *t*-tests. The values are means ± SEM. Statistically significant differences versus 6F/3D were determined at each temperature. *$P < 0.05$, **$P < 0.01$ ($n = 6$).

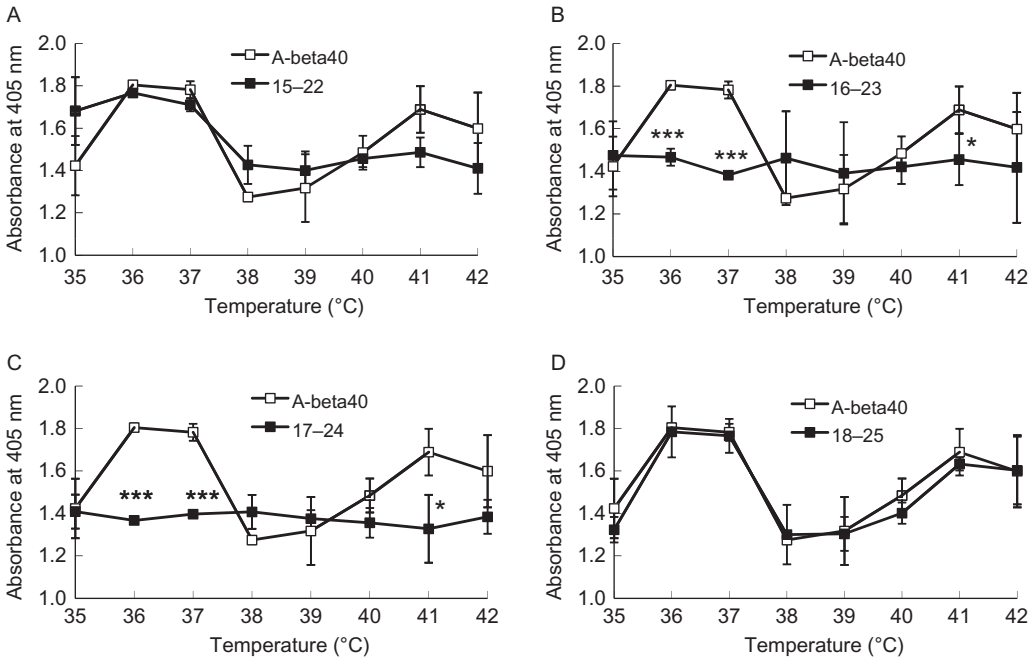


**Figure 2** Changes of antibody affinity towards temperature-modified Aβ40 in the presence of β-sheet breaker peptides (βSBPs). The reactivity of 6F/3D was determined by enzyme-linked immunosorbent assay for temperature-modified Aβ40 without βSBP (□) and with relevant βSBP (■): 15–22 (A), 16–23 (B), 17–24(C) and 18–25(D). Statistical comparisons were made with unpaired *t*-tests. The values are means ± SEM. Statistically significant differences versus Aβ40 alone were determined at each temperature. $*P < 0.05$, $***P < 0.001$ ($n = 6$).

different antibodies were similarly affected by various temperatures. The anti 1–7 polyclonal antibody exhibited the lowest reactivity against temperature-modified Aβ40, and this reactivity was significantly different from the other antibodies. The monoclonal anti 5–10 antibody showed high levels of reactivity compared with antibodies 6F/3D (anti 9–14) and anti 1–7, but not from 4G8 (anti 17–21). For both anti 5–10 and 1–7 antibodies, the reactivity was constant through the whole temperature ranges (35–42°C), and no temperature-dependent difference was detected. The monoclonal antibody 6F/3D showed temperature-dependent reactivity, and the reactivity was bimodal; decreasing and increasing reactivity of 0.5 and 0.4 absorbance units, respectively, when the modified temperature was increased from 36 to 38°C, and from 38 to 41°C respectively. On the other hand, 4G8 showed temperature-dependent reactivity; apparent decreased reactivity of 0.6 absorbance units when the temperature was increased from 36 to 40°C. No significant difference was detected between 6F/3D and 4G8. Thus, the 9–14 and 17–21 amino acid residues within the Aβ40 peptide were sensitive to temperature changes.

*Inhibition of temperature-modified conformational changes of Aβ40 by βSBP*

The preliminary study indicated that Aβ1–16 did not change the reactivity of Aβ40,whereas a change was evident for Aβ17–42 (data not shown). According to the present results shown in Figure 1, we investigated the reactivity of 6F/3D antibody towards the mixture of temperature-modified Aβ40 with βSBP 15–22, 16–23, 17–24 or 18–25. We chose 6F/3D, which recognized 9–14 of Aβ40, to avoid direct interaction with the βSBPs, because 4G8 recognized 17–21 of Aβ40 which includes the sequences of the βSBPs. The reactivity of Aβ40 alone was regarded as control. The βSBP 15–22 (Figure 2A) and 18–25 (Figure 2D) did not change the reactivity of Aβ40; however, βSBP 16–23 (Figure 2B) and 17–24 (Figure 2C) could change the reactivity of Aβ40 in a constant manner. In the present study, the molar ratio of Aβ40 to the βSBPs was 1:5, and a lower ratio of βSBPs to Aβ40 was not effective; however, higher ratios showed almost the same effects as those with the 1:5 molar ratio (data not shown). We repeated these assays using βSBPs made up in 0.4 M NaOH and adjusted to pH 7, the same conditions as in the CD studies, and measured the changes of antibody affinity towards temperature-modified Aβ40. The signals from Aβ40 with the βSBPs prepared in DMSO were almost the same as those with the βSBPs prepared in NaOH, and the immunoreactivity patterns were similar to each other (data not shown).

*Effect of βSBPs on temperature-induced conformational changes of Aβ40 in the presence and absence of PK*

The amount of protein remaining on the membrane before and after PK digestion at three different temperatures 36, 38 and 42°C, and at 20°C as a soluble Aβ40 control is shown in Figure 3A as a representative result of six experiments. The soluble control showed remarkable differences before and after PK digestion. No significant effect was detected for temperatures within each βSBP group; however, the spot density was lower in the presence of βSBPs 16–23 and 17–24 after PK digestion than Aβ40 alone with PK, indicating the effects of these βSBPs were to increase Aβ40 sensitivity to PK digestion. Figure 3B shows the average pixel density of each spot at 38°C

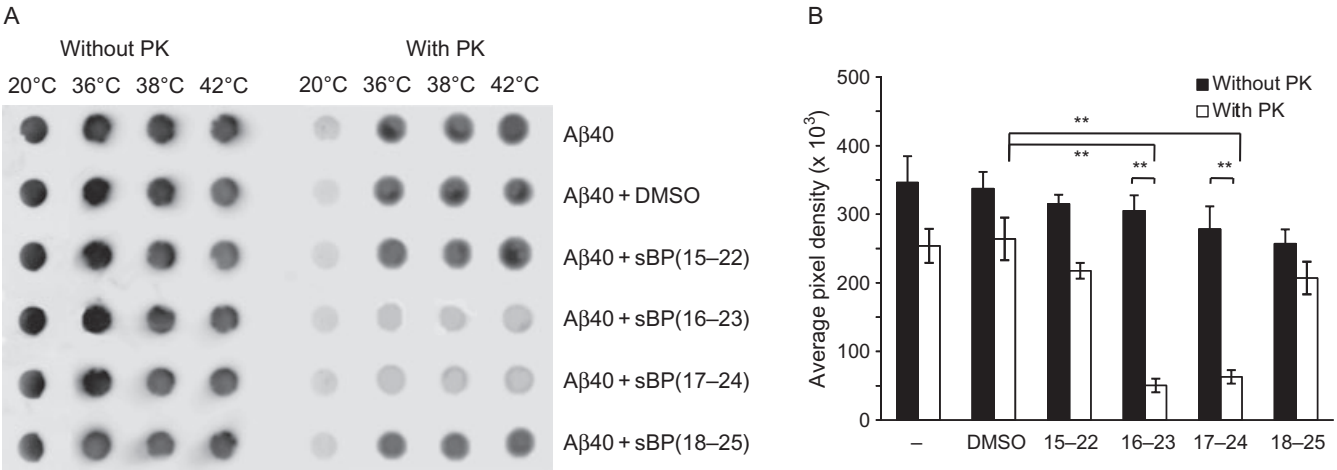


**Figure 3** Determination of protease K (PK) sensitivity for temperature-modified Aβ40 in the presence of β-sheet breaker peptides (βSBPs) by dot blot. Effects of βSBPs 15–22, 16–23, 17–24 and 18–25 on PK-induced digestion of temperature-modified Aβ40 at 36, 38, 42°C and at 20°C as a control were determined by dot blot. (A) Each spot shows the remaining Aβ40 without or with PK, and the figure is a representative of six experiments. (B) The average pixel density of each spot at 38°C was measured by NIH image analysis after subtracting the mean background pixel density from that of the spots. Values are means ± SEM. Statistical analysis was performed with analysis of variance and significant differences. $^{**}P < 0.01$ ($n = 6$).

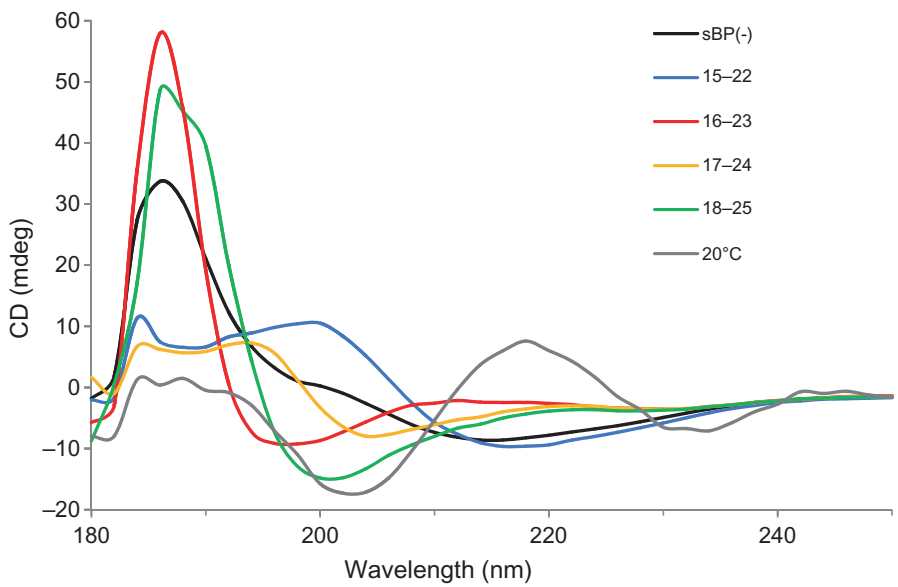


**Figure 4** Circular dichroism (CD) spectra reveal the reduction of thermally induced β-sheet formation of Aβ40 at 38°C with β-sheet breaker peptide (βSBP). The secondary structure of thermally induced Aβ40 at 38°C, and at 20°C as a control in the presence or absence of βSBP was measured by far-UV CD spectra. Spectra for Aβ40 alone (sBP) as a control, and mixtures with βSBP 15–22, 16–23, 17–24 and 18–25 are shown.

in Figure 3A. In this study, the PK digestion showed a tendency to reduce Aβ40 spot density (30%), but this was not significant, and βSBPs 15–22 and 18–25 did not alter the spot density level either. On the other hand, the βSBPs 16–23 and 17–24 reduced the spot densities by around 80% after PK digestion, indicating greater digestion with PK of Aβ40 and higher PK sensitivity in the presence of the two βSBPs. The spots of Aβ40 with/without DMSO before and after PK digestion showed no differences, indicating DMSO at this concentration did not affect the results. These results are also comparable with the results of the ELISA. In the present study, we also tested various molar ratios of Aβ40 to the βSBPs at 1:1, 1:5, 1:10 and 1:20 for PK digestion, and observed no altered PK sensitivity below the molar ratio of 1:5.

*CD spectra of Aβ40 with βSBPs*

The CD experiments were carried out to confirm the effects of βSBPs to prevent temperature-induced conformational changes of Aβ40 at 38°C (Figure 4). The minimum CD spectrum of Aβ40 alone was at around 218 nm, which corresponds to a β-sheet-rich conformation. Although the minimum CD spectrum of Aβ40 with βSBP 15–22 was not shifted, the spectra of Aβ40 with βSBP 16–23, 17–24 and

18–25 were shifted to around 200 nm, which was close to the soluble control Aβ40 at 20°C. In addition, the CD amplitudes at 218 nm of Aβ40 with βSBP 16–23, 17–24 and 18–25 were increased more than the values for Aβ40 alone by 3.9–5.8 mdeg. These results indicate that all βSBPs except 15–22 could inhibit β-sheet formation of Aβ40 at 38°C.

## Discussion

It is generally accepted that the conformational changes within the Aβ protein that result in aggregation of aberrant β-sheet-rich intermediates, are important in the development of AD. Determination of the sequences within Aβ that are involved in these changes, and inhibition of such changes by βSBPs have considerable potential for a novel therapeutic approach to AD.

The conformational changes are induced by thermodynamic stress (Sengupta *et al.*, 2003). However, various physiological factors including pH shift, co-precipitants of metal ions (Atwood *et al.*, 1998) and abnormal oxidative metabolites including cholesterol-derived aldehydes (Bieschke *et al.*, 2005) are also involved in the conformational changes of Aβ40 in brain.

High temperature (including fever) could induce structural changes in Aβ (tangles and plaques) or changes in brain similar to those observed in AD (Sinigaglia-Coimbra *et al.*, 2002). Our preliminary experiments revealed that when a wide range of temperatures (0–99°C) was applied, the conformation of Aβ40 at 0–20°C was (α-helical, whereas conformational changes of Aβ40 towards β-sheet configuration were observed at 35–45, 60–65 and 80–85°C. The occurrence of changes within specific temperature ranges may indicate thermal specificity or the adoption by Aβ40 of various conformations at wide range of heating, due to increasing intermolecular β-sheet structures (Chu and Lin, 2001).

We chose to work over 35–42°C, a temperature range that includes the physiological limits, and found that the apparent changes at 36–38°C involved the epitopes around amino acid residues 9–14, whereas the changes induced at 36–40°C involved those around residues 17–21. We infer from these results that the 6F/3D epitope (amino acid residues 9–14) in Aβ40 was inaccessible at 38°C, and again exposed at around 41°C; however, that of the 4G8 epitope was inaccessible over 38°C. Both sequences have been reported to be involved in pH-induced conformational transitions of Aβ42 (Matsunaga *et al.*, 2002). However, CD spectra study for thermally modified Aβ40 at 36–40°C lacks conformational changes (data not shown), indicating retention of the secondary structure and only a minor loosening of the tertiary structure, within this temperature range.

Various terminuses and segments of Aβ40 may display different biophysical properties and biological activities. The C-terminus of Aβ40 quiescent fibrils lacks β-sheet structure compared to the more rigid structure within the 24–30 segment (Williams *et al.*, 2006). It seems that the thermal changes take place in a part of Aβ40 involving the central hydrophobic region that is also implicated in various biological functions including interaction with other proteins (Golabek *et al.*, 1996). Moreover, the 9–21 sequence includes amino acid residues 10–23 that provide the structural basis of the hydrophobic behaviour under physiological conditions (Hilbich *et al.*, 1991).

It has been reported that the pentapeptides βSBP KLVFF (16–20) and LPFFD (17–21 analogous) interact with the main Aβ structure via hydrogen bridges with the βSBP binding in the plane of the amyloid dimer (Hetenyi *et al.*, 2002). The present results from ELISA and dot blot studies showed that temperature-induced conformational changes were reversed by octapeptide βSBPs 16–23 and 17–24, but not by βSBPs 15–22 and 18–25.

As the βSBPs are not water soluble, we could not avoid the use of DMSO, NaOH being the alternative for dissolving the βSBPs. The use of DMSO may alter Aβ40 conformation; however, a pH shift by NaOH may have a greater effect on Aβ40 behaviour (Matsunaga *et al.*, 2002). In the present study, we used minimal amount of DMSO to dissolve the βSBPs, and diluted with water to the final DMSO concentration of 0.2% (Figures 2 and 3), which did not affect the conformation of Aβ40 (Shen and Murphy, 1995; Kanaoka *et al.*, 2003), but this concentration is high enough to change the cell membranes and induce heat shock proteins in biological experiments.

However, as traces of DMSO disturb the CD spectra, we used 0.4 M NaOH instead of DMSO to dissolve the βSBPs and adjusted to pH 7. The effect of βSBP 18–25 revealed by CD study did not correspond to the results of ELISA and dot blot (Figure 4). We speculate that incubation of Aβ40 with pure water and a pH shift by NaOH to dissolve the βSBPs in the CD study may be responsible for the different results of βSBP 18–25 between ELISA, dot blot and CD studies.

The region 16–23 (KLVFFAED) used in this study contains KLVFF that has been reported to protect against Aβ toxicity (Pallitto *et al.*, 1999). Moreover, it has been proposed that the region 1–16 is not active by itself, but is required for the activity of Aβ40, whereas the region 29–42 is inactive in pH-induced conformational changes (Matsunaga *et al.*, 2004). From the results obtained in this study, we suggest that the amino acid residues 16–24 within Aβ40 is the region that is involved in reversal of temperature-dependent conformational changes by the βSBPs. However, our studies showed that the βSBPs 16–23 and 17–24 prevented the temperature-dependent conformational changes of Aβ40.

Our results also showed that βSBP 16–23 and 17–24 exposed Aβ40 to the activity of PK, as PK did not affect the already denatured Aβ40, but could digest it in the presence of βSBPs 16–23 and 17–24, but not the βSBPs 15–22 and 18–25. The ability of partial Aβ fragments around Aβ 16–23 to inhibit Aβ40 aggregation may be due to their ability to bind the central hydrophobic region of Aβ40 including the temperature-sensitive region, thereby destabilizing oligomers necessary for fibril stability. As a result, the site of protease cleavage would be exposed to the activity of PK. However, the βSBP 15–22 besides not inhibiting temperature-conformational changes, did not reduce PK digestion.

In conclusion, our results revealed that Aβ40 exhibited differential temperature-dependent conformational changes: the epitopes 9–14 involved in the conformational change induced at 36–38°C, whereas epitope 17–21 involved in those induced at 36–40°C. These changes could be reversed by the βSBPs 16–23 and 17–24. These βSBPs could be of value in the

treatment of AD, and *in vivo* studies are required to confirm the possible therapeutic value of the compounds.

## Acknowledgements

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