Biochemical and Biophysical Research Communications 439 (2013) 252–257

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Two β-strands of RAGE participate in the recognition and transport of amyloid-β peptide across the blood brain barrier

Sun-Jick Kim [a,1], Jang-Won Ahn [a,1], Hee Kim [b], Hee-Jin Ha [b], Sang-Woo Lee [b], Hyun-Kuk Kim [c], Sangho Lee [a], Hyun-Seok Hong [b], Young Ho Kim [b,*], Cheol Yong Choi [a,*]

[a] Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[b] Medifron_DBT Co., Ltd., 349 Sandanro, Ansan 425-839, Republic of Korea
[c] Advanced Protein Technologies Corp., 864-1 Iui-dong, Suwon 443-766, Republic of Korea

ARTICLE INFO

*Article history:*
Received 8 August 2013
Available online 23 August 2013

*Keywords:*
RAGE
Aβ
Alzheimer's disease
β-Strand

ABSTRACT

Amyloid-β (Aβ) peptide is central to the development of brain pathology in Alzheimer disease (AD) patients. Association with receptors for advanced glycation end-products (RAGE) enables the transport of Aβ peptide from circulating blood to human brain, and also causes the activation of the NF-κB signaling pathway. Here we show that two β-strands of RAGE participate in the interaction with Aβ peptide. Serial deletion analysis of the RAGE V domain indicates that the third and eighth β-strands are required for interaction with Aβ peptide. Site-directed mutagenesis of amino acids located in the third and eighth β-strands abolish the interaction of RAGE with Aβ peptide. Wild-type RAGE activates the NF-κB signaling pathway in response to Aβ peptide treatment, while a RAGE mutant defective in Aβ binding does not. Furthermore, use of peptide for the third β-strand or a RAGE monoclonal antibody that targets the RAGE–Aβ interaction interface inhibited transport of the Aβ peptide across the blood brain barrier in a mice model. These results provide information crucial to the development of RAGE-derived therapeutic reagents for Alzheimer disease.



## 1. Introduction

Amyloid-β (Aβ) peptide is central to the development of brain pathology in Alzheimer disease (AD) patients [1,2]. Aβ is produced by cleavage of APP by β- and γ-secretase in the plasma membrane of neuronal cells and deposited outside of neuronal cells to form pathogenic plaques. Alternatively, Aβ is transported in and out of the human brain by receptors for advanced glycation end-products (RAGE) and LRP, respectively [3,4]. RAGE-mediated transport of circulating Aβ across the blood brain barrier (BBB) is an important factor in the pathogenesis of cerebrovascular β-amyloidosis [3,5].

RAGE is a member of the immunoglobulin super family with an extracellular domain that consists of a variable region as well as two constant regions which resemble immunoglobulin [6]. RAGE was believed to be a pattern recognition receptor, because there is no common structure between RAGE ligands [7]. NMR studies of the RAGE motif involved in interaction with S100B, a RAGE ligand, indicate that multiple amino acids covering the surface of the RAGE V domain are determinant for association with S100B [8]. X-ray crystallography of the RAGE extracellular ectodomain indicated that the RAGE V domain is composed of eight β-strands and one short α-helical structure [9]. In addition, hydrophobic patches are located mainly at the back sheet of the β-strands. Moreover, a positively charged surface was uncovered at the front sheet of the β-strands [8]. S100B conformation changes upon $Ca^{2+}$-binding and hydrophobic patches are exposed following conformational change [8]. The exposed hydrophobic patch of the S100B dimer associates with hydrophobic patches of RAGE for interaction [8]. On the other hand, AGE appears to recognize the positively charged surface of RAGE which is organized by basic amino acids of the front sheet of the β-strands [10,11].

Deciphering how Aβ interacts with RAGE and characterizing the motif(s) involved in the interaction can provide insight into understanding the role of RAGE in Aβ transport and Aβ-mediated induction of the inflammatory response through RAGE. We identified the RAGE domain responsible for interaction with Aβ. Serial deletion and point mutation analysis of RAGE indicated that two β-strands of RAGE participate in the interaction with Aβ. These results provide information crucial to the understanding of Aβ–RAGE interaction and the development of therapeutic small molecules for the treatment of AD.

* Corresponding authors. Fax: +82 31 290 7015 (C.Y. Choi).
*E-mail addresses:* yhkim@medifron.com (Y.H. Kim), choicy@skku.ac.kr (C.Y. Choi).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.08.047

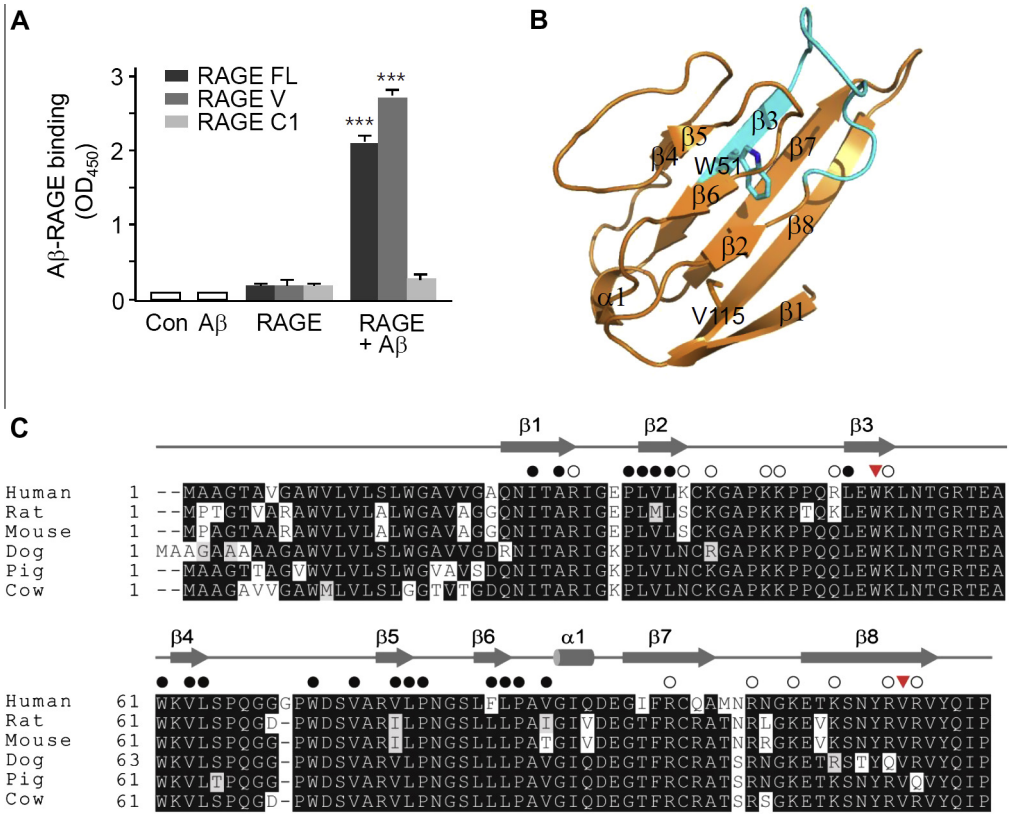


**Fig. 1.** Aβ(1-42) interacts with the V domain of RAGE. (A) Aβ(1-42) peptide was incubated with immobilized full-length, V domain, or C1 domain of sRAGE, followed by incubation with HRP-conjugated anti-Aβ antibody. RAGE–Aβ binding was quantitated by measuring $OD_{450}$. Data were analyzed by ANOVA with Tukey-multiple comparisons tests (***$p < 0.001$). (B) Ribbon representation of crystal structure of human RAGE V domain (PDB ID: 3CJJ; ref [9]) generated by PyMOL. The β-strands are numbered 1 through 8, beginning at the N-terminus of the RAGE V domain. Note that there is one α-helix (α1) between β6 and β7. Amino acid residues from 38 to 52 are colored in cyan, which are deleted in the M2 deletion mutant. (C) Multiple sequence alignment of the RAGE V domain from human (*Homo sapiens*), rat (*Rattus norvegicus*), mouse (*Mus musculus*), dog (*Canis lupus familiaris*), pig (*Sus scrofa*) and cow (*Bos Taurus*). The conserved residues are colored with a black background. The start and end point of each β-strand are indicated by an arrow on top of the aligned amino acid sequences. Residues located on the positively charged surface and hydrophobic patches are denoted as open or closed circles above the sequences, respectively. Mutated residues are indicated by red inverse triangles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## 2. Materials and methods

### 2.1. Plasmid construction

The pET-sRAGE plasmid encoding human sRAGE between an N-terminal His-Tag and C-terminal Avi-Tag containing a biotinylation motif was described previously [12]. The Myc-RAGE expression plasmid was constructed by insertion of PCR-amplified DNA fragments into the EcoR1 and Xba1 sites of pCS3MT. Other sRAGE deletion mutants (M1–M5 and D1–D3) and point mutants of sRAGE were inserted into the XhoI and EcoRI sites of pCS3MT. Point mutants (W51P, V115P, and WPVP double mutant) were generated using the Muta-Direct™ Site-Directed Mutagenesis Kit (iNtRON, #15071). Mutations were verified by DNA sequencing. The expression plasmid encoding the GST-Aβ fusion protein was constructed by insertion of four copies of the DNA fragment encoding Aβ(1-42) into the BamHI and SalI sites of pGEX-4T-2.

### 2.2. Luciferase assay

Mouse hippocampal neuronal cell line HT22 cells were grown in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum. For luciferase reporter assays, HT22 cells seeded into 6-well plates were transfected with the RAGE expression plasmid (1 μg), and the NF-κB-Luc reporter plasmid (1 μg), where the luciferase gene is under the control of NF-κB binding sites. The total amount of transfected plasmid was adjusted using empty vectors. Thirty-six hours after transfection, cells were seeded again onto 96-well plates and treated with Aβ peptides for 18 h. Luciferase activity was measured using the Luciferase Reporter Assay System (Promega) and a Genios luminometer (TECAN, Austria). Transfection efficiency was normalized against β-galactosidase expression. Each experiment was repeated at least three times.

### 2.3. GST pull-down assay

The GST-Aβ fusion proteins were expressed in *Escherichia coli* BL21 (DE3) cells, and purified with glutathione-Sepharose beads. Various Myc-RAGE deletion mutants were synthesized by using a TNT coupled *in vitro* transcription-translation system (Promega, # L4600). Synthesized proteins were incubated with GST-Aβ at RT for 30 min in GST binding buffer (20 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1.5 mM $MgCl_2$ 0.05% NP-40) and washed three times with phosphate buffered saline containing 0.5% Triton X-100, followed by immunoblotting with anti-Myc antibody. For peptide inhibition, equal amounts (3 ng) of β3 (RLEWKL) or β8 (ETKSNYRVRV) peptide were included in the reaction.

### 2.4. Protein expression and purification

The pET-sRAGE and pBirA plasmids were introduced into Origami (DE3). The transformed cells were grown in LB broth, and

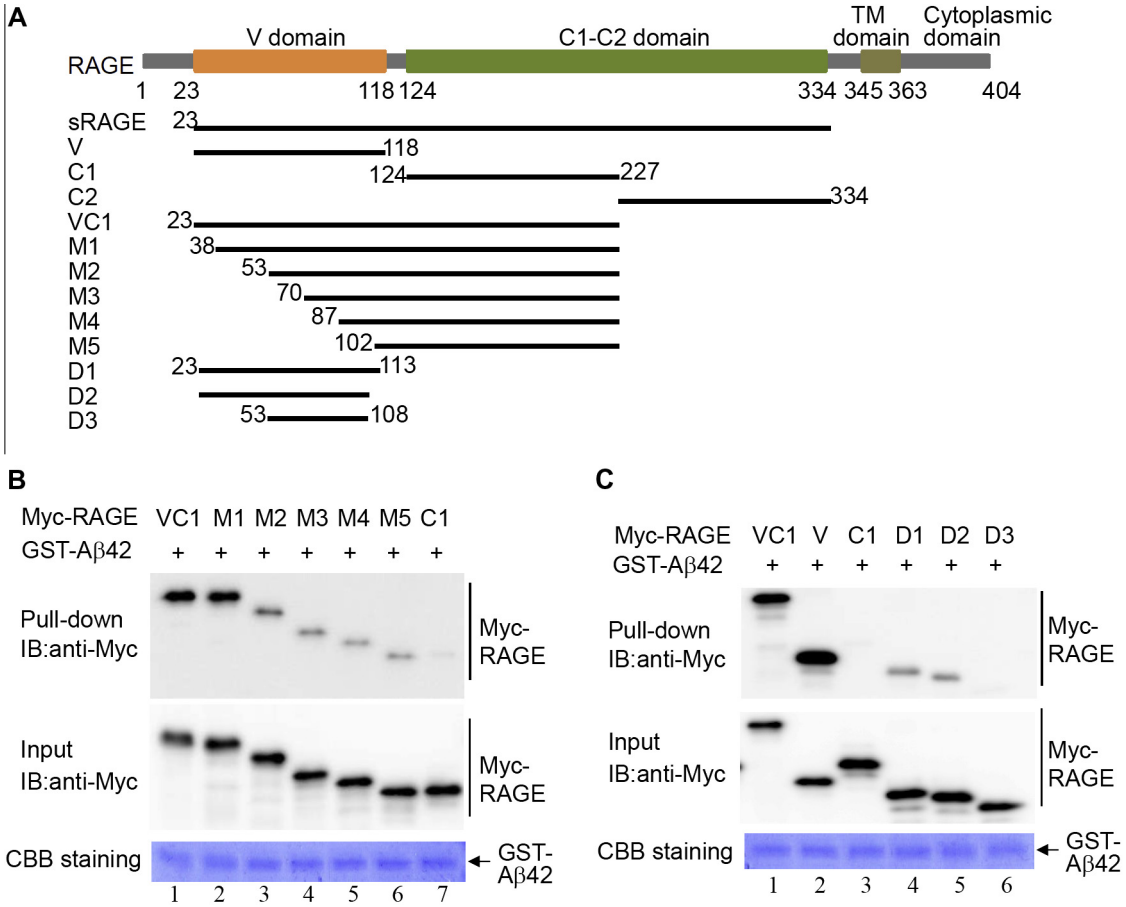


**Fig. 2.** Analysis of RAGE domains involved in interaction with Aβ. (A) Schematics of the RAGE deletion mutants utilized in the GST pull-down assay are shown. (B and C) Various Myc-RAGE deletion mutants were translated *in vitro* and subjected to GST pull-down analysis using GST-Aβ42. Bound proteins were eluted and resolved by 10% SDS–PAGE, and detected by immunoblotting using anti-Myc antibody. Input denotes 5% of proteins used in the analysis.

expression and biotinylation of the target protein was induced by adding 1 mM IPTG and 50 mM d-biotin for 18 h at 25 °C. The biotinylated His-RAGE was purified by chromatography using nickel–nitrilotriacetic acid (Ni–NTA) resin (HiTrap™ Chelating HP, #71-7005-00 AX).

### 2.5. Immunoprecipitation

Full-length Myc-hsRAGE and deletion mutants (VC1, V or C1 domain of RAGE) were synthesized by using a TNT coupled *in vitro* transcription–translation system (Promega, # L4600). Myc-hsRAGE was incubated overnight with anti-RAGE antibody and protein A/G-Sepharose beads at 4 °C on a rotating wheel. Immunoprecipitates were analyzed by immunoblotting using anti-Myc antibody. Mouse monoclonal anti-RAGE antibody targeting the peptide sequence (PWDSVARVLP) was generated by Abmart (http://www.ab-mart.com/).

### 2.6. ELISA

One microgram of purified biotinylated-human RAGE and 1 μL of 10 μM Aβ solution in 100 μL of TBS-T with 2.5% BSA were incubated on a streptavidin-coated plate for 60 min. After washing the plate with TBS-T, horseradish-peroxidase conjugated 4G8 antibody was added into each well to detect the bound Aβ. The plate was incubated for 60 min at ambient temperature. After washing with TBS-T, the plate was developed with TMB substrate, the reaction was stopped with sulfuric acid. Absorbance was read on a Sunrise plate reader (TECHAN) at 450 nm.

### 2.7. Acute model study in wild-type mice

ICR mice were obtained from SAMTACO (Korea). The test IgG (1 and 10 mg protein/200 μl PBS) or β3 peptide (N-RLEWKL-C) was injected into the tail vein of 3 month-old ICR mice (25 g, $n = 4$, male) and allowed to circulate for 10 min. 200 μL of 25 μM human Aβ(1-42) in PBS was injected into the tail vein and allowed to circulate for 30 min. Postmortem mice brains were removed immediately after sacrifice. Brain samples were homogenized in RIPA buffer (100 mM Tris, pH 8.0, 150 mM NaCl, 0.5% DOC, 1% NP-40, 0.2% SDS and proteinase inhibitor cocktail) and sonicated for 20 s on ice. The Aβ levels were analyzed using ELISA kits (Cat# 27711, IBL). All procedures for animal tests were approved by the Committee on the Ethics of Animal Experiments of Medifron Co. Ltd. in South Korea (Permit Number: AEC-20080509-0001 permitted by Korea FDA). All surgical procedures were performed with care to minimize pain and discomfort.

## 3. Results

### 3.1. Analysis of the RAGE domain involved in interaction with Aβ

The receptor for advanced glycation end-products (RAGE) is the main receptor for transporting amyloid-beta peptide (Aβ) across the blood–brain barrier (BBB) from blood to brain. To further

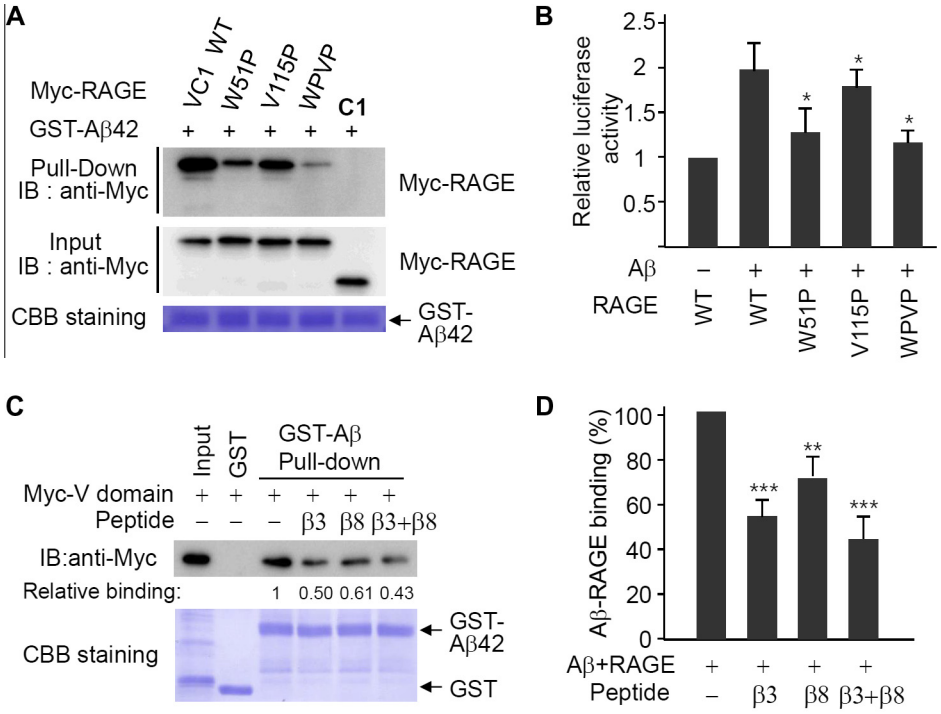


**Fig. 3.** Analysis of RAGE mutants for interaction with Aβ and activation of the NF-κB pathway. (A) RAGE point mutants were translated *in vitro* and subjected to GST pull-down analysis using GST-Aβ42. Bound proteins were eluted and resolved by 10% SDS–PAGE, and detected by immunoblotting using anti-Myc antibody. Input denotes 5% of proteins used in the analysis. The blot stained with Coomassie Brilliant Blue shows GST-Aβ proteins used in the pull-down assay. (B) HT22 cells were transfected with a NF-κB reporter plasmid and wild-type or point mutated (W51P, V115P, or WPVP) RAGE expression plasmids 36 h prior to Aβ(1-42) treatment. Eighteen hours after Aβ treatment, luciferase activity was measured. Transfection efficiency was normalized against β-galactosidase expression. Experiments were repeated at least three times. Error bars indicate the standard error of the means of three independent experiments. Data were analyzed by ANOVA with Dunnett's multiple comparisons tests ($^{*}p < 0.05$). (C) Myc-RAGE V domain was translated *in vitro* and subjected to GST pull-down analysis using GST-Aβ42 in the absence or presence of β3 or β8 peptide. Bound proteins were eluted and resolved by 10% SDS–PAGE, and detected by immunoblotting using anti-Myc antibody. Input denotes 5% of proteins used in the analysis. The blot stained with Coomassie Brilliant Blue shows GST and GST-Aβ proteins used in the pull-down assay. (D) Aβ(1-42) peptide was incubated with immobilized full-length sRAGE, in the absence or presence of β3 or β8 peptide, followed by incubation with HRP-conjugated anti-Aβ antibody. RAGE–Aβ binding was quantitated by measuring $OD_{450}$. Experiments were repeated at least three times. Error bars indicate the standard error of the means of three independent experiments. Data were analyzed by ANOVA with Dunnett's multiple comparisons tests ($^{***}p < 0.001$; $^{**}0.001 < p < 0.01$).

understand RAGE-mediated Aβ transport at the molecular level, we analyzed RAGE domains for interaction with Aβ. Expression plasmids for human sRAGE and its deletion mutants (V, C1, and C2 domain of RAGE) were constructed in which a poly-histidine tag is located at the N-terminal of RAGE and a biotinylation motif fused in frame to the C-terminus of RAGE. The binding of RAGE to Aβ was analyzed with an enzyme-linked immunosorbent assay (ELISA) using anti-Aβ antibody. Colorimetric observance was detected only when both Aβ and sRAGE were present in the reaction, but not Aβ or RAGE alone, indicating that Aβ specifically binds to sRAGE. In addition, the RAGE V domain, but not the C1 domain, bound to Aβ, consistent with a previous report [13,14], indicating that Aβ binds to the V domain of RAGE (Fig. 1A). Analysis of the crystal structure of RAGE indicated that the V domain of RAGE consists of eight β-strands and one α-helical structure intervening between the sixth and seventh β-strand [8,9] (Fig. 1B). In addition, amino acids throughout the entire RAGE V domain determine surface properties such as hydrophobic or positively charged patches (Fig. 1C), which are important for interactions with S100B and advanced glycation end-products (AGE) [8,11]. We hypothesized that Aβ recognizes a specific motif rather than surface electrostatics, since Aβ was reported to recognize two β-strands of α1-antichymotrypsin [15]. To determine whether Aβ requires a specific β-strand or electrostatic surface, a series of deletion mutants were constructed and utilized in a GST pull-down assay using GST-Aβ (Fig. 2A). Pull-down analysis indicated that N-terminal deletion to amino acid 53 decreased interaction of RAGE with Aβ. In addition, further deletion to amino acid 124 completely abrogated RAGE–Aβ interaction (Fig. 2B). These results indicated that both the third and eighth β-strands of RAGE participate in the interaction of RAGE with Aβ. To verify this result, deletion mutants of the V domain were generated. Consistent with the above results, removal of five C-terminal amino acids (C-terminal deletion from amino acid 114 to 118) reduced RAGE–Aβ interaction (Fig. 2C, lane 4). Further deletion of the RAGE N-terminal to amino acid 52 completely abrogated the interaction between RAGE and Aβ Fig. 2C, lane 6). These results clearly demonstrate that both the third and eighth β-strands of RAGE participate in the recognition of Aβ.

### 3.2. Mutational analysis of RAGE for interaction with Aβ

To further determine the structural requirements of RAGE in Aβ binding, site-directed mutagenesis was performed. To disrupt the structure of the β-strands, Trp51 in the third β-strand and/or Val115 in the eighth β-strand were substituted to prolines. GST pull-down analysis using these RAGE point mutants indicated that single substitutions (W51P or V115P) reduced binding of RAGE to GST-Aβ. Moreover, double substitution (WPVP; W51P and V115P) abrogated the interaction between RAGE and GST-Aβ (Fig. 3A). These results demonstrated that two β-strands of RAGE contribute to the recognition of Aβ. Activation of the NF-κB signaling pathway is a cellular response to the association of Aβ with RAGE. To determine whether the NF-κB signaling pathway is affected by RAGE mutants, wild-type or point mutants of full-length RAGE were transiently transfected into HT22 cells together with an NF-κB reporter plasmid, and the cells were exposed to Aβ for 18 h. Forced

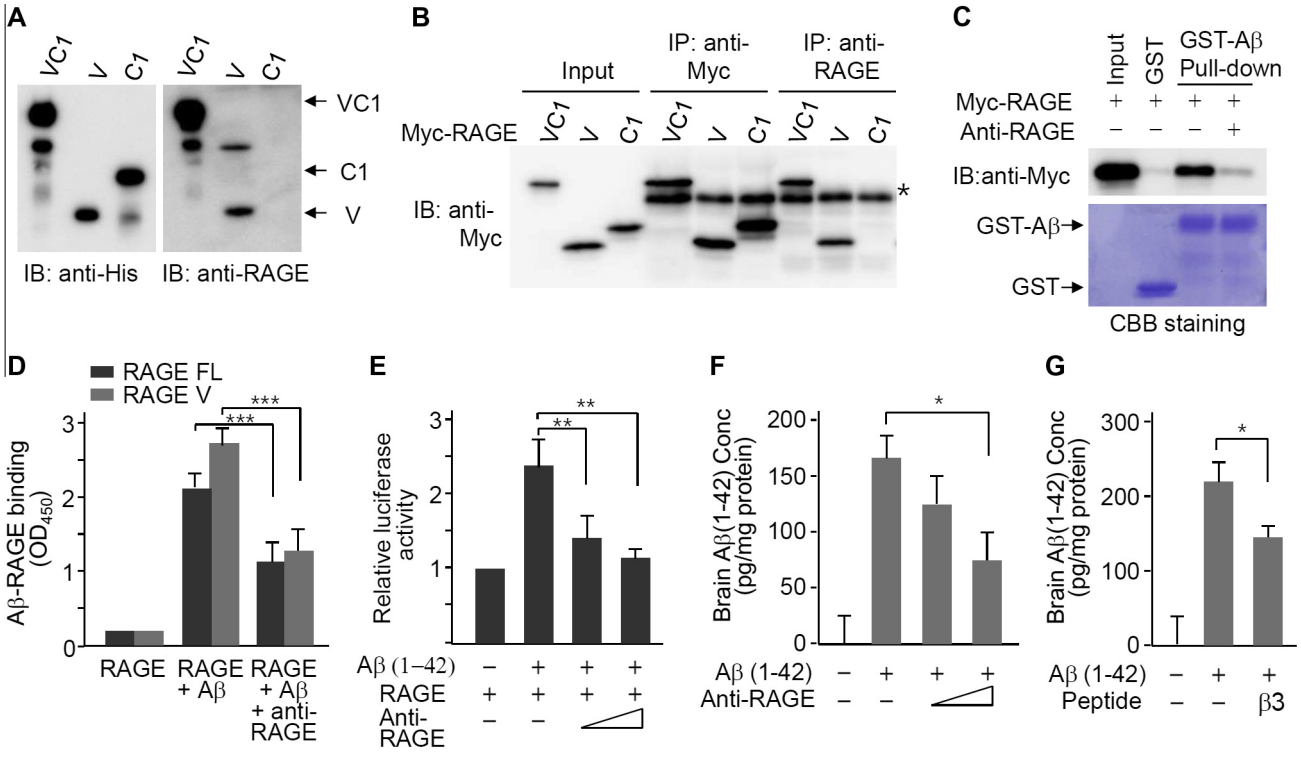


**Fig. 4.** Blockage of RAGE–Aβ interaction inhibits Aβ transport across the blood brain barrier and Aβ-mediated activation of the NF-κB pathway. (A) Affinity-purified RAGE proteins (VC1, V, or C1 domain of RAGE) were subjected to immunoblotting using anti-RAGE monoclonal antibody targeting RAGE V domain. (B) *In vitro* translated Myc-RAGE (VC1, V, or C1 domain of RAGE) proteins were immunoprecipitated with anti-RAGE monoclonal antibody and anti-Myc antibody as a positive control. The immunoprecipitated proteins were analyzed by immunoblotting with HRP-conjugated anti-Myc antibody. The asterisk indicates a non-specific band. (C) Myc-RAGE was translated *in vitro* and subjected to GST pull-down analysis using GST-Aβ42. Myc-RAGE was incubated with GST-Aβ in the presence or absence of anti-RAGE antibody as indicated. The bound proteins were eluted and resolved by 10% SDS–PAGE, and detected by immunoblotting using anti-Myc antibody. Input denotes 5% of proteins used in the analysis. The blot stained with Coomassie Brilliant Blue shows GST and GST-Aβ proteins used in the pull-down assay. (D) Aβ(1-42) peptide was incubated with immobilized full-length, V domain, or C1 domain of sRAGE in the absence or presence of anti-RAGE monoclonal antibody, followed by incubation with HRP-conjugated anti-Aβ antibody. RAGE-Aβ binding was quantitated by measuring $OD_{450}$. $^{***}P < 0.001$ by ANOVA with Tukey-multiple comparisons tests. (E) HT22 cells were transfected with a NF-κB reporter plasmid and wild-type RAGE expression plasmids 36 h prior to Aβ(1-42) treatment. The transfected cells were treated with increasing amounts of anti-RAGE antibodies 30 min prior to Aβ(1-42) treatment. Eighteen hours after Aβ treatment, luciferase activity was measured using the Luciferase assay system (Promega). Transfection efficiency was normalized against β-galactosidase expression. Experiments were repeated at least three times. Error bars indicate the standard error of the means of three independent experiments. $^{**}P < 0.01$ by ANOVA with Tukey-multiple comparisons tests. (F and G) Human Aβ(1-42) peptide was injected into the mouse tail vein and transport of plasma Aβ(1-42) to brain was measured by quantitation of human Aβ(1-42) in sacrificed mouse brain extracts ($n = 4$). The anti-RAGE monoclonal antibody or β3 peptide was injected 30 min prior to injection of Aβ(1-42) peptide. $^{*}P < 0.05$ by ANOVA with Tukey-multiple comparisons tests (F) and paired Student's *t* test (G).

expression of RAGE promoted activation of NF-κB signaling pathway in response to administration of Aβ (Fig. 3B). However, expression of RAGE single mutants reduced activation of the NF-κB pathway and the RAGE double mutant further decreased activation of the NF-κB target reporter in response to Aβ (Fig. 3B). These results indicate that two β-strands of RAGE are involved in RAGE-mediated signaling cascade for NF-κB activation. To verify that the two β-strands of RAGE are involved in interaction with Aβ, peptides corresponding to the third and eighth β-strands were used to compete with Aβ recognition of RAGE. As shown in Fig. 3C, the interaction of RAGE with GST-Aβ was inhibited efficiently by the β3 and β8 peptides. Consistently, the binding of Aβ to full-length sRAGE was blocked by the β3 and β8 peptides in ELISA (Fig. 3D). These results indicate that the third and eighth β-strands of RAGE are the motifs for interaction with Aβ.

### 3.3. Inhibition of Aβ transport and NF-κB activation via a monoclonal antibody against RAGE

In order to validate the RAGE domains for Aβ interaction in an animal model and to develop a potential inhibitor which disrupts RAGE–Aβ interaction, anti-RAGE monoclonal antibodies were generated and one was selected for further study. The RAGE V domain was recognized in immunoblotting (Fig. 4A) and was specifically immunoprecipitated with the anti-RAGE antibody (Fig. 4B), verifying that the monoclonal antibody recognizes the RAGE V domain. To determine whether this anti-RAGE antibody targeting the RAGE V domain inhibits RAGE–Aβ interaction, a GST pull-down assay and ELISA were carried out in the presence of anti-RAGE antibody. As shown previously, *in vitro* translated Myc-RAGE bound well to GST-Aβ, but the interaction was inhibited by addition of anti-RAGE antibody (Fig. 4C, lane 4). This result was confirmed in ELISA using full-length sRAGE and Aβ(1-42) peptide. Incubation of Aβ(1-42) peptide with immobilized sRAGE resulted in RAGE–Aβ interaction, and this interaction was inhibited by addition of anti-RAGE antibody (Fig. 4D). These results indicated that the RAGE–Aβ interaction can be blocked by an anti-RAGE antibody targeting the RAGE V domain. To examine the functional relevance of the RAGE–Aβ interaction through the V domain, we determined whether RAGE-mediated NF-κB activation and Aβ transport across the brain BBB are affected by the anti-RAGE monoclonal antibody targeting the RAGE V domain. Administration of HT22 cells with Aβ peptide activated transcription from a NF-κB reporter plasmid, but the activation was gradually inhibited in the presence of the anti-RAGE antibody in a dose-dependent manner (Fig. 4E). To examine the effects of anti-RAGE antibody on Aβ transport across the BBB, Aβ(1-42) peptide was injected into the mouse tail vein and transport of plasma Aβ(1-42) to brain was measured by quantitation of Aβ(1-42) from sacrificed mouse brain extracts. As shown in Fig. 4F, transport of the Aβ(1-42) peptide across the BBB was inhibited by injection of the anti-RAGE monoclonal antibody tar-

geting the RAGE V domain in a dose-dependent manner. Moreover, Aβ transport across the BBB was also inhibited by injection of the β3 peptide of RAGE V domain (Fig. 4G). These results demonstrated that blocking the RAGE–Aβ interaction with a V domain-specific anti-RAGE antibody and β3 peptide of RAGE V domain impairs RAGE-mediated NF-κB activation and Aβ transport from plasma to brain.

## 4. Discussion

In this study, we found that two β-strands of RAGE, and not surface properties, participate in the recognition of Aβ. The surface properties of RAGE are determined by the composition of amino acids in the entire V domain, not by specific motifs. Hydrophobic patches are mainly located at the back sheet of the β2, β5 and β6 strands (closed circles in Fig. 1C), and the positively charged surfaces are organized by loops of the front sheet of β-strands (open circles in Fig. 1C). Therefore, RAGE–Aβ interaction appears to be mediated by specific bipartite β-strands rather than by hydrophobic patches or positively charged surfaces. In support of this idea, each RAGE point mutant defective in Aβ interaction was generated by substitution of the Trp51 or Val115 residue, which are not associated with hydrophobic patches or positively charged surfaces. Notably, both peptides for the third and eighth β-strands markedly inhibited interaction between RAGE and Aβ (Fig. 3). Taken together, it is more likely that Aβ recognizes bipartite β-strands of RAGE rather than patterns of the surface of RAGE such as positively charged or hydrophobic patches. Aβ interaction with α1-antichymotrypsin (ACT) shows a similar mode of bimodal binding [15], providing further support for our conclusions regarding Aβ–RAGE interaction. The serpin ACT is a major component of brain amyloid plaques in Alzheimer's disease. Aβ was shown to bind to ACT through insertion of amino- and carboxyl-terminal Aβ segments into the sA and sC β-strands of ACT, respectively. The bimodal binding of Aβ to ACT results in reciprocal conformational changes. Aβ binding leads to loss of ACT inhibitor activity [15], and ACT can either stimulate formation of the fibrillar form of Aβ peptide [16,17] or destabilize preformed Aβ fibrils [18,19], depending on the stoichiometry of ACT to Aβ. Similarly, interaction of RAGE with Aβ also inhibits polymerization of Aβ even after prolonged periods of incubation [20]. Aβ is composed of β-sheets and RAGE binds β-sheet fibrillar material regardless of the composition of the subunits, such as amyloid A and amylin [21]. Further studies would provide insight into common structural motifs in RAGE ligands. Finding common structural motifs such as specific β-strands of RAGE which are functionally associated with human disease would be beneficial in the development of therapeutic strategies for the treatment of Alzheimer's disease.

## Acknowledgments

We thank Sachiko Machida (National Food Research Institute, Japan) for providing pET-sRAGE and pBirA plasmids. This work was supported by the Collaborative Research Program among industry, academia and research institutes through the Korea Industrial Technology Association (KOITA) funded by the Ministry of Education, Science and Technology (KOITA-2011-2-17 to CYC) and by a Korea Science and Engineering Foundation (KOSEF) grant (KRF-2008-313-C00605 and the Ubiquitome Research Program, 2012-0006126 to CYC).

## References

[1] J. Hardy, A hundred years of Alzheimer's disease research, Neuron 52 (2006) 3–13.
[2] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide, Nat. Rev. Mol. Cell Biol. 8 (2007) 101–112.
[3] R. Deane, B.V. Zlokovic, Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease, Curr. Alzheimer Res. 4 (2007) 191–197.
[4] R. Deane, Z. Wu, B.V. Zlokovic, RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier, Stroke 35 (2004) 2628–2631.
[5] S.H. Han, Y.H. Kim, I. Mook-Jung, RAGE: the beneficial and deleterious effects by diverse mechanisms of actions, Mol. Cells 31 (2011) 91–97.
[6] M. Neeper, A.M. Schmidt, J. Brett, S.D. Yan, F. Wang, Y.C. Pan, K. Elliston, D. Stern, A. Shaw, Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins, J. Biol. Chem. 267 (1992) 14998–15004.
[7] J. Xie, S. Reverdatto, A. Frolov, R. Hoffmann, D.S. Burz, A. Shekhtman, Structural basis for pattern recognition by the receptor for advanced glycation end products (RAGE), J. Biol. Chem. 283 (2008) 27255–27269.
[8] H. Park, F.G. Adsit, J.C. Boyington, The 1.5 A crystal structure of human receptor for advanced glycation endproducts (RAGE) ectodomains reveals unique features determining ligand binding, J. Biol. Chem. 285 (2010) 40762–40770.
[9] M. Koch, S. Chitayat, B.M. Dattilo, A. Schiefner, J. Diez, W.J. Chazin, G. Fritz, Structural basis for ligand recognition and activation of RAGE, Structure 18 (2010) 1342–1352.
[10] S. Matsumoto, T. Yoshida, H. Murata, S. Harada, N. Fujita, S. Nakamura, Y. Yamamoto, T. Watanabe, H. Yonekura, H. Yamamoto, T. Ohkubo, Y. Kobayashi, Solution structure of the variable-type domain of the receptor for advanced glycation end products: new insight into AGE–RAGE interaction, Biochemistry 47 (2008) 12299–12311.
[11] J. Xue, V. Rai, D. Singer, S. Chabierski, J. Xie, S. Reverdatto, D.S. Burz, A.M. Schmidt, R. Hoffmann, A. Shekhtman, Advanced glycation end product recognition by the receptor for AGEs, Structure 19 (2011) 722–732.
[12] M. Kumano-Kuramochi, Q. Xie, Y. Sakakibara, S. Niimi, K. Sekizawa, S. Komba, S. Machida, Expression and characterization of recombinant C-terminal biotinylated extracellular domain of human receptor for advanced glycation end products (hsRAGE) in *Escherichia coli*, J. Biochem. 143 (2008) 229–236.
[13] E. Sturchler, A. Galichet, M. Weibel, E. Leclerc, C.W. Heizmann, Site-specific blockade of RAGE-Vd prevents amyloid-beta oligomer neurotoxicity, J. Neurosci. 28 (2008) 5149–5158.
[14] R. Deane, I. Singh, A.P. Sagare, R.D. Bell, N.T. Ross, B. LaRue, R. Love, S. Perry, N. Paquette, R.J. Deane, M. Thiyagarajan, T. Zarcone, G. Fritz, A.E. Friedman, B.L. Miller, B.V. Zlokovic, A multimodal RAGE-specific inhibitor reduces amyloid beta-mediated brain disorder in a mouse model of Alzheimer disease, J. Clin. Invest. 122 (2012) 1377–1392.
[15] S. Janciauskiene, H. Rubin, C.M. Lukacs, H.T. Wright, Alzheimer's peptide Abeta1-42 binds to two beta-sheets of alpha1-antichymotrypsin and transforms it from inhibitor to substrate, J. Biol. Chem. 273 (1998) 28360–28364.
[16] S. Janciauskiene, S. Eriksson, H.T. Wright, A specific structural interaction of Alzheimer's peptide A beta 1–42 with alpha 1-antichymotrypsin, Nat. Struct. Biol. 3 (1996) 668–671.
[17] J. Ma, H.B. Brewer Jr., H. Potter, Alzheimer A beta neurotoxicity: promotion by antichymotrypsin, ApoE4; inhibition by A beta-related peptides, Neurobiol. Aging 17 (1996) 773–780.
[18] P.E. Fraser, J.T. Nguyen, D.R. McLachlan, C.R. Abraham, D.A. Kirschner, Alpha 1-antichymotrypsin binding to Alzheimer A beta peptides is sequence specific and induces fibril disaggregation in vitro, J. Neurochem. 61 (1993) 298–305.
[19] S. Eriksson, S. Janciauskiene, L. Lannfelt, Alpha 1-antichymotrypsin regulates Alzheimer beta-amyloid peptide fibril formation, Proc. Natl. Acad. Sci. USA 92 (1995) 2313–2317.
[20] M.O. Chaney, W.B. Stine, T.A. Kokjohn, Y.M. Kuo, C. Esh, A. Rahman, D.C. Luehrs, A.M. Schmidt, D. Stern, S.D. Yan, A.E. Roher, RAGE and amyloid beta interactions: atomic force microscopy and molecular modeling, Biochim. Biophys. Acta 1741 (2005) 199–205.
[21] S.D. Yan, H. Zhu, A. Zhu, A. Golabek, H. Du, A. Roher, J. Yu, C. Soto, A.M. Schmidt, D. Stern, M. Kindy, Receptor-dependent cell stress and amyloid accumulation in systemic amyloidosis, Nat. Med. 6 (2000) 643–651.