Biologically Active Fragment of the Receptor for Advanced Glycation End Products (RAGE) Is Able to Inhibit Oligomerization of the Beta-Amyloid

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Abstract — It was found earlier that the synthetic fragment corresponding to the 60–76 sequence of the extracellular domain of the receptor for advanced glycation end products (RAGE) had a protective effect on animal and cellular models of Alzheimer’s disease. It was proposed that this effect was mediated via the interaction of the peptide with beta-amyloid (Aβ), which was one of the RAGE ligands, by inhibiting the formation of toxic Aβ oligomers. The aim of this study was an application of physicochemical methods to an investigation of the ability of the 60–76 peptide to prevent the Aβ40 oligomerization in solution in comparison with the nonprotective 65–76 truncated peptide. The dynamics of the formation of the Aβ40 fibrils in the presence of the peptides was evaluated using thioflavin T. The relative sizes of oligomers were determined by dynamic light scattering. The peptide binding to Aβ40 was examined by fluorescence titration. We demonstrated by the two methods that the peptide corresponding to the 60–76 sequence of RAGE considerably inhibited (by more than 90%) the formation of oligomers and fibrils of Aβ40 distinct from the 65–76 peptide. In addition, we found that the protective effect of the peptides and their ability to inhibit the Aβ40 oligomerization did not correlate with their binding to the monomeric/tetrameric Aβ40. We confirmed in vitro the hypothesis that the protective activity of the synthetic 60–76 fragment of RAGE was associated with its ability to inhibit the Aβ oligomerization.

Keywords: receptor for the advanced glycation end products, synthetic peptides, Alzheimer’s disease, beta-amyloid, oligomerization, dynamic light scattering, thioflavin T

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INTRODUCTION

The process of an oligomerization and formation of fibrils of beta-amyloid (Aβ) is known to occur in the human brain during Alzheimer’s disease (AD) and play a key role in the development of neurodegeneration. The fibrils form deposits along with the tau-protein [1–5], whereas soluble oligomers whose sizes are smaller than those of fibrils are associated with the Aβ neurotoxicity [6–8]. This fact has been demonstrated both in vitro and in vivo [15]. Note that the oligomerization and the fibril formation are results of the pathological accumulation of Aβ in the brain. One of the pathways of the Aβ transport from the bloodstream to the brain is mediated by the receptor of the advanced glycation end products (RAGE) [17]. RAGE is located on membranes of the endothelium, neurons, astrocytes, and microglia [18–21]. This receptor is over-expressed during the AD development, and Aβ is excessively accumulated in the brain [18, 20, 22, 23]. Aβ is one of the RAGE ligands which activate pathogenic signal pathways in microglia [24, 25] that stimulate general negative effects, such as an uncontrollable inflammation, mitochondrial dysfunction, oxidative stress, and neuronal damage [18, 26, 27]. Thus, RAGE is able to mediate the neurotoxic effect of Aβ, i.e., accelerate development of the cognitive disorders and pathological changes in neurons [28]. RAGE binds all forms of Aβ, including monomers, oligomers, and
fibrils. In addition, it was shown with the use of the antibody panel to RAGE that various Aβ forms were bound to different sites of the receptors depending on the level of the RAGE oligomerization. In particular, the Aβ fibrils are bound to the C2 domain, whereas the soluble oligomers interact with domain V [29].

Previously, we demonstrated that the synthetic peptide corresponding to the 60–76 sequence of the RAGE V-domain exhibited the therapeutic effect after intranasal administration to the animals subjected to OB. This peptide prevented the spatial memory impairment in the OB-mice and the 5xFAD transgenic mice. Both mouse lines were the AD models in which the mice had behavioral, biochemical, and morphological symptoms of Alzheimer’s disease [30, 31]. The peptide administration also prevented both the Aβ accumulation in the brain and the pathological changes in neurons [32]. Earlier, the ability of the 60–76 peptide to eliminate the toxic effects of Aβ in the mixed primary culture of neurons and astrocytes was also demonstrated [33]. The 60–76 peptide was shown to specifically activate RAGE, resulting in the release of glutamate mainly from astrocytes [34]. The indirect data on a possibility of the peptide interaction with Aβ were obtained after the intranasal administration of the fluorescent analog to the 5xFAD mice. As a result, a colocalization of the peptide and the amyloid plaques was observed in the brain sections [32]. The peptide was also shown to bind Aβ in solution [33, 35]. Taking into account all the data, we proposed that peptide protective activity could be mediated through its interaction with Aβ and the subsequent inhibition of the formation of toxic oligomers.

The goal of this study was an investigation of the ability of the protective synthetic peptide corresponding to the 60–76 sequence of RAGE to prevent the Aβ oligomerization in solution with the use of two physicochemical methods: dynamic light scattering and the analysis of the peptide binding to the thioflavin T fluorescent dye.

**RESULTS AND DISCUSSION**

Sequences of the 60–76 peptide (P1) and its shortened 65–76 fragment (P2), selected for comparison, exhibited no activity in the animal model of AD [32, 36] are given in Table 1. We used Aβ40 that was one of the two main alloforms of Aβ which were present in the amyloid plaques [37, 38]. Solubility of Aβ40 was much higher and the rate of its oligomerization was lower than that of the Aβ42 isoform. Therefore, we could prepare aqueous solutions with the exact Aβ40 concentration and have time for measurements by laboratory devices [39]. The particle size in the Aβ40 solution after its aggregation and the influence of the P1 and P2 peptides on this aggregation were examined by dynamic light scattering (DLS). At the same time, the ability of the peptides to inhibit the formation of protofibrils and fibrils of Aβ40 in solution was analyzed according to a change in the fluorescence of thioflavin T. In addition, the ability of the P1 and P2 peptides to directly bind Aβ40 was studied by fluorescent titration.

**Determination of sizes of the Aβ40 oligomers in solution by dynamic light scattering.** We used the DLS method for studies of the influence of the RAGE fragments on the Aβ40 oligomerization. First, we determined sizes of the Aβ40 molecules in the starting solutions which were prepared by various methods. The size distributions of the particles in the solution of disaggregated Aβ40 and Aβ40 that was dissolved in water without a preliminary disaggregation are shown in Figs. 1a and 1b.

Two pools of particles were observed in the solution of the disaggregated Aβ40 (Fig. 1a). The first pool involved small particles with an average hydrodynamic diameter of 0.9 nm. This value corresponded to the diameter of monomers. The second pool contained particles with an average diameter of 3.8 nm (dimers, trimers, and tetramers of Aβ40) [40]. No small particles were found in the non-disaggregated solution of Aβ40, suggesting the absence of monomers, dimers, trimmers, and tetramers. Instead, a pool with a wide spectrum of the particle sizes (from 8 to 80 nm) was observed (Fig. 1b). In addition, a small number of large particles with a diameter of 200–2000 nm were present. These particles were highly oligomeric molecules. The disaggregated Aβ40 was used for the further studies (Fig. 1a).

The solution of disaggregated Aβ40 with the peptides or without them was further subjected to aggrega-
tion, i.e., the solution was incubated under conditions that facilitated the aggregation. The sizes of all the Aβ40 molecules in the mixture were determined by dynamic light scattering (Fig. 2).

The size distribution analysis of the particles confirmed the occurrence of the Aβ40 oligomerization. Only one pool of aggregates with the average value of the hydrodynamic diameter of 2500 nm was found in the Aβ40 solution without the peptides (Fig. 2a). The size distribution of the particles in solution was dramatically changed after the Aβ40 incubation in the presence of the P1 peptide. Two pools of small particles which were similar to those in Fig. 1a were observed in Fig. 2b, suggesting the presence of the large number of small particles (1–6 nm in diameter) in the mixture. In addition, two small pools of larger aggregates with diameters of 500 and 1100 nm were present. Nevertheless, these aggregates were much smaller than those in the solution of the “pure” Aβ40. The size distribution of the particles in solution after the Aβ40 incubation with the P2 peptide was analogous to that for the Aβ40 solution without the peptide. Only one pool of very large aggregates with an average diameter of 1500 nm was presented on the graph. This diameter is slightly lower than that for the pure Aβ40, but considerably higher than that for the mixture of Aβ40 with the P1 peptide (Fig. 2c). No small particles remained in the solution. Thus, we demonstrated using dynamic light scattering that the P1 peptide significantly inhibited the Aβ40 oligomerization and prevented the formation of large aggregates, whereas the P2 peptide had almost no influence on the oligomerization process.

Investigation of Aβ40 fibrillogenesis in the presence of the RAGE fragments by changing the fluorescence of thioflavin T. The next stage of our investigation was an examination of an ability of the RAGE peptides to influence the formation of Aβ40 fibrils. They were large oligomers of a prolate structure which were located in the amyloid plaques in the brains of AD patients. The dynamics of the Aβ40 oligomerization in the presence of the two peptides was studied by fluorescent spectrophotometry using thioflavin T dye (ThT). ThT allowed detection of the formation of the β-structure that was specific for the protofibrils and fibrils of Aβ. Before the buffer addition, Aβ40 was dis-

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**Fig. 1.** The size distribution of the oligomers from the initial Aβ40 solution that was determined by the DLS method: (a), disaggregated Aβ40; (b), non-disaggregated Aβ40.
aggregated in the same way as in other studies, so that the oligomer content in the beginning of the experiment was at the lowest level. Previously, we demonstrated that the synthetic Aβ40 that was used in this experiment was able to form the characteristic β-pleated fibrils of beta-amyloid in aqueous solutions [41].

The fluorescence values of Aβ40 in the presence of the P1 and P2 peptides were compared with the fluorescence of Aβ40 in the absence of the peptides (Fig. 3). The experimental data gave a typical curve of the amyloid aggregation with the ten-hour lag-phase in which the fluorescence remained unchanged (the nucleation phase). Then, the six-hour elongation phase followed. In this phase, the formation of the amyloid protofibrils and fibrils manifested itself as an increase in ThT fluorescence. The evident existence of the lag-phase pointed to the absence of the amyloid fibrils in our ini-

Fig. 2. The size distribution of the Aβ40 oligomers after their 40-hour incubation that was determined by the DLS method: (a), only Aβ40; (b), Aβ40 with P1; (c), Aβ40 with P2.
cent titration demonstrated that both peptides formed for the P2 peptide (Fig. 4b). The results of the fluorescence for the P1 peptide (Fig. 4a) and weaker. It should be noted that only more than 90%. The effect of the P2 peptide was much influence of the P1 and P2 peptides on the Aβ oligomerization in solution by the P1 peptide that exhibited the protective effect on the animal and cellular modes of AD. At the same time, the inactive shortened 65–76 fragment of RAGE (the P2 peptide) barely affected the Aβ40 oligomerization. Simultaneously, the experiments with the ThT use demonstrated that the P1 peptide inhibited a formation of the Aβ40 fibrils by more than 90%. The effect of the P2 peptide was much weaker. It should be noted that only β-structures of the fibrils could be identified by ThT fluorescence, in contrast to DLS analysis which allowed identification of the whole spectrum of the oligomers. Therefore, only the DLS method reflected an impartial difference in the P1 and P2 ability to inhibit the process of the Aβ oligomerization.

Fluorescent titration of the RAGE fragments using Aβ40. We tried to find an explanation of the different influence of the P1 and P2 peptides on the Aβ40 oligomerization and studied the direct ability of both peptides to bind Aβ. We performed the fluorescent titration of both peptides using Aβ40. Tryptophan residue of the peptides changed its fluorescence after the peptide binding to a ligand, and, thus, this residue was a convenient fluorescent source during the titration [42]. The tryptophan presence in the peptides prevented the introduction of a fluorescent label, preserving the native peptide properties. The concentrations of the P1 peptide with two tryptophan residues and the P2 peptide with one tryptophan residue were 0.5 and 2 μM (in order to intensify the signal), respectively. The Aβ40 solution was preliminarily prepared under the conditions which minimized the oligomer content in the solution as described in the previous experiments. The initial Aβ40 concentration was 200 nM. The results of the fluorescence experiments were presented as a graph of the 1/ΔF ratio in comparison with 1/[S], where [S] was the Aβ40 concentration in the cuvette, ΔF was a difference between the intensities in the absence and in the presence of the definite Aβ40 concentration (Fig. 4).

The dissociation constants were $K_d = 1.41 \pm 0.29 \, \text{μM}$ for the P1 peptide (Fig. 4a) and $K_d = 0.51 \pm 0.08 \, \text{μM}$ for the P2 peptide (Fig. 4b). The results of the fluorescent titration demonstrated that both peptides formed complexes with Aβ40, and the P2–Aβ40 complex was even more stable than that with P1, according to the calculated $K_d$ values. Evidently, the method for the fluorescent titration could not explain the different ability of the peptides to inhibit the Aβ40 oligomerization. It is interesting that Kamynina et al. [33] showed that P2 did not bind to the Aβ40 according to the fluorescent titration, whereas the $K_d$ value for P1 was almost identical to that in our experiment. This fact could probably be explained by the absence of the Aβ40 disaggregation procedure in the paper [33]. P2 possibly formed complexes only with the monomers-tetramers of Aβ40, but could not bind the oligomers. At the same time, P1 could be bound to any form of Aβ40. These results suggested that the peptide binding to the monomers-tetramers of Aβ40 was insufficient for the inhibition of the formation of the large oligomers and fibrils. Possibly, the peptide binding to the small and medium oligomers was also required for the inhibition of the oligomerization.

Fig. 3. Kinetics of the formation of the Aβ40 fibrils.

All the results of this study are summarized in Table 1. The previously obtained data on the testing of the spatial memory of mice that were subjected to the olfactory bulbectomy (OB) after the intranasal administration of P1 or P2 are given in the last column of Table 1 [32]. As follows from Table 1, only P1 exhibited the protecting activity. P1 inhibited the process of the Aβ oligomerization, and this P1 ability was demonstrated by two methods: the change in the ThT fluorescence and by DLS. At the same time, the inhibition of the Aβ oligomerization by the P1 and P2 peptides did not correlate with the peptide binding to the monomeric/tetrameric Aβ40.
**EXPERIMENTAL**

**Peptide synthesis.** The fragments of the human RAGE (Q15109, UniProtKB/SwissProt) and human Aβ40 (P05067.3, UniProtKB/Swiss-Prot) were synthesized by the solid phase method on the Wang polymer according to the Fmoc/But-strategy and purified by HPLC as described previously [32, 41]. The peptide homogeneity was >95%.

**Disaggregation of the (1–40) beta-amyloid.** The lyophilized synthetic Aβ40 was dissolved in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) (99%; Sigma-Aldrich, United States) in a concentration of 1 mg/mL, and the solution was divided to aliquots. HFIP was evaporated in a steam of gaseous argon. The peptide film was dissolved in DMSO (Sigma-Aldrich, United States) to the final concentration of 20 mg/mL. This solution was vigorously shaken and kept at –20°C for 12 h before the buffer addition.

**Formation of the oligomers and fibrils of Aβ40.** The buffer (20 mM Tris-HCl and 100 mM NaCl, pH 7.4, 1850 μL) was added to the solution of Aβ40 (250 μg) in DMSO (12.5 μL), and the mixture was immediately shaken for 30 s. For the preparation of the mixture of Aβ40 and one of the RAGE fragments, P1 (200 μg) or P2 (140 μg) were dissolved in the buffer (1850 μL) and added to the solution of Aβ40 (250 μg) in DMSO. The mixture was shaken for 30 s. The samples were aged at 37°C for 40 h.

**Dynamic light scattering.** The dynamic light scattering (DLS) was measured on a Zetasizer Nano ZS analyzer (Malvern Instruments Ltd., Great Britain) equipped with a laser source with λ = 633 nm with detection at a scattering angle of 173°. The samples contained the disaggregated Aβ40 or the disaggregated Aβ40 that was incubated with the peptides or without them under the conditions of formation of the oligomers and the fibrils. The prepared solution (100 μL) was placed in the DLS cuvette for 20 min. The data on light scattering were analyzed for an evaluation of the size distribution of the particles and their z-averaged hydrodynamic diameter (d_h) using the Stokes–Einstein equation that suggested the spherical shape of the particles.

**Analysis of the Aβ40 fibrillogenesis with an application of thioflavin T.** The formation of the Aβ40 fibrils was observed according to binding of thioflavin T [43] on a Clariostar Plus fluorescent flatbed reader (BMG LABTECH, Germany). The solution of Aβ40 or Aβ40 in the mixture with one of the peptides (75 μL) was taken at a designated time and mixed with the 50-μM solution of ThT (25 μL) in wells of a plate that was made from the black polystyrene (SPL) for the ThT fluorescence measurements. The final concentrations of Aβ40, the peptides, and ThT were 25, 50, and 12.5 μM, respectively. The sample fluorescence was measured at λ_excitation = 450 nm and λ_emission = 486 nm. At each time point, four aliquots were taken from every solution and placed in the wells of the plate. The ThT fluorescence was measured for every well no less than four times at different heights. The background buffer fluorescence was subtracted. The average value and the standard error of the mean (SEM) were calculated for every point.

**Fluorescent titration.** The fluorescent titration of the synthetic peptides was performed as described previously [33]. The concentrations of the P1 peptide and P2 peptide were 0.5 and 2 μM, respectively. The disaggregated Aβ40 was dissolved in DMSO and added to the peptide solutions in the increasing concentrations: 200, 400, 800, 1200, 1600, 2000, 2500, 3000, 4000, and

![Fig. 4. The fluorescent titration of the peptides using Aβ40: (a) P1 in a concentration of 0.5 μM; (b) P2 in a concentration of 2 μM. Curves of the change in the intensity of the tryptophan fluorescence (1/ΔF) depending on the 1/[S] concentration of Aβ40 at a wave length of 520 nm. The results of one of the five independent experiments were shown.](image)
CONCLUSIONS

We demonstrated in this study that the biologically active synthetic peptide corresponding to the 60–76 sequence of RAGE affected the oligomerization process of Aβ. The method for the dynamic light scattering and the analysis of the thioflavin fluorescence showed that the 60–76 peptide, which exhibited a protective effect on the spatial memory of mice in the experimental models of Alzheimer’s disease, considerably inhibited the formation of the oligomers and fibrils of Aβ40. The 65–76 inactive shortened fragment of the peptide was devoid of this ability. At the same time, the inhibition of the Aβ oligomerization by the 60–76 and 65–76 peptides and their protective activity did not correlate with the peptide binding to the monomeric/tetrameric Aβ40.

Thus, we confirmed the hypothesis that the protective activity of the synthetic peptide corresponding to the 60–76 sequence of RAGE was associated with its ability to inhibit the formation of the toxic oligomers of Aβ. One of the possible ways for producing the protective effect of the 60–76 peptide, which was promising for therapy of Alzheimer’s disease, was elucidated.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants and animals performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Glenner, G.G. and Wong, C.W., Biochem. Biophys. Res. Commun., 1984, vol. 120, pp. 885–890. https://doi.org/10.1016/s0006-291x(84)80190-4
2. Hardy, J., Proc. Natl. Acad. Sci. U.S.A., 1997, vol. 94, pp. 2095–2097. https://doi.org/10.1073/pnas.94.6.2095
3. Hardy, J.A. and Higgins, G.A., Science, 1992, vol. 256, pp. 184–185. https://doi.org/10.1126/science.1566067
4. Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., Mcdonald, B.L., and Beyreuther, K., Proc. Natl. Acad. Sci. U.S.A., 1985, vol. 82, pp. 4245–4249. https://doi.org/10.1073/pnas.82.12.4245
5. Selkoe, D.J., J. Neuropathol. Exp. Neurol., 1994, vol. 53, pp. 438–447. https://doi.org/10.1097/00050072-199409000-00003
6. Gandy, S., Simon, A.J., Steele, J.W., Lublin, A.L., Lah, J.J., Walker, L.C., Levey, A.I., Krafft, G.A., Levy, E., Checher, F., Glabe, C., Bilker, W.B., Abel, T., Schmeidler, J., and Ehrlrich, M.E., Ann. Neurol., 2010, vol. 68, pp. 220–230. https://doi.org/10.1002/ana.22052
7. Kayed, R. and Lasagna-Reeves, C.A., in Alzheimer’s Disease: Advances for a New Century, vol. 3, Perry, G., Avila, J., Zhu, X., Smith, M.A., and Sorensen, A., Eds., Amsterdam: IOS Press, 2013, pp. 67–78. https://doi.org/10.3233/978-1-61499-154-0-67
8. Klein, W.L., Krafft, G.A., and Finch, C.E., Trends Neurosci., 2001, vol. 24, pp. 219–224. https://doi.org/10.1010/s00422-001-01749-5
9. Harper, J.D., Wong, S.S., Lieber, C.M., and Lansbury, P.T., Chem. Biol., 1997, vol. 4, pp. 119–125. https://doi.org/10.1016/s1074-5521(97)90255-6
10. Hartley, D.M., Walsh, D.M., Ye, C.P.P., Diehl, T., Vasquez, S., Vassilev, P.M., Teplow, D.B., and Selkoe, D.J., J. Neurosci., 1999, vol. 19, pp. 8876–8884. https://doi.org/10.1523/Jneurosci.19-20-08876.1999
11. Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E., Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., and Klein, W.L., Proc. Natl. Acad. Sci. U.S.A., 1998, vol. 95, pp. 6448–6453. https://doi.org/10.1073/pnas.95.11.6448
12. Nilsberth, C., Westlind-Danielsson, A., Eckman, C.B., Condron, M.M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D.B., Younkin, S.G., Naslund, J., and Lannfelt, L., Nat. Neurosci., 2001, vol. 4, pp. 887–893. https://doi.org/10.1038/nn0901-887
13. Oda, T., Wals, P., Osterburg, H.H., Johnson, S.A., Paisnetti, G.M., Morgan, T.E., Rozovsky, I., Stine, W.B., Snyder, S.W., Holzman, T.F., Krafft, G.A., and Finch, C.E., Exp. Neurol., 1995, vol. 136, pp. 22–31. https://doi.org/10.1006/exnr.1995.1080
