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Amyloid-β containing isoaspartate 7 as potential biomarker and drug target in Alzheimer's disease

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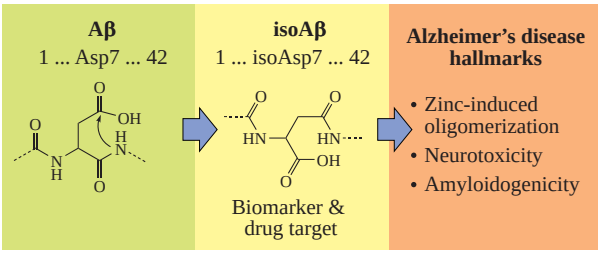
Recent data about structure, interaction with biometals, aggregation state, proteolysis by angiotensin converting enzyme, neurotoxicity and amyloidogenicity of amyloid-β containing isoaspartate 7 (isoAsp7-Aβ) in comparison with intact amyloid-β are summarized. Possible role of isoAsp7-Aβ as a prospective biomarker for molecular diagnostics and drug target in anti-amyloid therapy of Alzheimer's disease is discussed.



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Alzheimer's disease (AD), first described in 1906,[1] is currently the most common neurodegenerative proteopathy in the world, affecting over 44 million people.[2] By 2050 it is assumed that 100 million patients will be diagnosed with AD. In Russia, the number of such patients is about one million.[3] The clinical signs of AD in terms of psychiatry include slow but steady weakening of mental abilities and socio-cultural skills of the patient for 3–10 years after manifestation of the first case of inadequate behavior in everyday life (sudden unprovoked aggression, unreasonable emotions, 'loss' of space-time borders, short-term amnesia, and other). The disease is accompanied by organic destruction of the brain, which ultimately leads to the patient's death from respiratory failure.

Hereditary variants of AD constitute less than 1% of all cases of this pathology and are associated with gene mutations leading to the constant excess over physiological level of amyloid-β (Aβ), which is a short 39–43 amino acid polypeptide, heterogeneous at the C-terminus.[4] The causes of sporadic AD variants, which cover more than 95% of the patients remain unknown, but are closely related to abnormal aggregation of the endogenous Aβ. There are three main neuromorphological hallmarks that definitively confirm (posthumously) the diagnosis for all AD variants: (1) the presence in specific brain regions of the characteristic extracellular aggregates (so-called amyloid plaques), whose main components are different isoforms of Aβ and ions of biometals (zinc, copper, iron); (2) the presence of neurofibrillary tangles (mainly formed by hyperphosphorylated tau protein); (3) degeneration of neurons.[5]

At present only symptomatic treatment for AD is available, directed against neurotransmitter deficiency and slowing down for 1–3 years the transition of average patient from relatively independent to a completely helpless condition. Five drugs approved by the US Food and Drug Administration (FDA) are used throughout the world and include four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, galantamine) and one NMDA receptor antagonist (memantine).[6] Tacrine has been approved by the FDA in 1993, donepezil in 1996, rivastigmine in 1998, galantamine in 2001, and memantine in 2003. From 2003 there were no new drugs for AD released to the global market, however strategies for their discovery based on the fundamental mechanisms of AD genesis are being actively developed.

Here we debate the critical role of Asp7 isomerization in altering the structural and functional properties of Aβ, which are associated with AD pathogenesis. The data presented in this review substantiate the use of Aβ with isomerized Asp7 (isoAβ) as a biomarker and drug target in the diagnostic and treatment of AD.

Factors of the Aβ pathological aggregation (zinc ions, chemical modifications)

According to the widely accepted 'amyloid hypothesis', conversion of the physiologically normal Aβ from monomeric state to soluble neurotoxic oligomers and subsequently to insoluble polymeric aggregates which eventually accumulate as amyloid plaques, serves as a triggering process of the AD pathogenesis.[7] Consequently prevention of cerebral β-amyloidogenesis by inhibiting pathological oligomerization of Aβ (the so-called anti-amyloid therapy) is considered as the most promising strategy for the treatment of AD.[8] A necessary prerequisite for the formation of aggregates is dimerization of Aβ monomers. Recently it was found that the Aβ dimers are neurotoxic and their concentration in the blood of patients with Alzheimer's disease correlates with clinical manifestations of the disease.[9,10] Thus blocking dimerization of Aβ appears to be the most effective way to prevent β-amyloidogenesis. Since Aβ in normal conformation is not a pathogenic molecule, this suggests the influence of other factors on the initiation of pathological cascade in AD.

The driving forces of Aβ pathological aggregation remain unknown, however it has been established that zinc ions play critical role in this process.[11] Human Aβ binds zinc ion through its metal-binding domain 1–16 ($Aβ_{1–16}$)[12,13] (Figure 1). This domain also contains the 11–14 segment responsible for zinc-induced dimerization of Aβ[14] leading to the formation of stable Aβ aggregates with beta-parallel arrangement of the monomers.[15] Notably, in amyloid plaques the amino acid residues 17–42 of Aβ ($Aβ_{17–42}$) form the structure composed of beta-sheets and turns, which form hydrophobic core, whereas residues 1–16 are located outside of this core and do not take part in stabilizing the structure of the modelled amyloid plaques.[16] The hydrophobic segment $Aβ_{17–42}$ though does not form amyloid aggregates *in vivo*,[17,18] pointing at the key role of the metal-binding domain 1–16 for cerebral β-amyloidogenesis in AD.[19]

A plausible hypothesis suggests that interaction of intracellular Aβ with genomic DNA can contribute to AD pathogenesis through interference with the normal DNA expression.[20] Indeed, localization of the intracellular Aβ was found in cell nuclei and binding of Aβ to DNA was demonstrated *in vitro*.[21,22] The Aβ–DNA interaction produces conformational changes in DNA. Potential activity of Aβ as a transcription factor *per se* has also been suggested based on selective interactions of Aβ with the promoter region of p53 gene and with regions of some AD-associated genes.[23] We have shown that the metal-binding domain of the Aβ peptide serves as a zinc-dependent DNA-binding site.[24] Binding of Aβ to DNA is induced by Zn^{2+} ions and is accelerated by the zinc-mediated oligomerization of the domain.

Capability of the Aβ aggregates that were extracted postmortem from the brain of AD patients to initiate cerebral β-amyloidogenesis was first demonstrated in experiments on monkeys subjected to intracerebral injection of corresponding material obtained by autopsy.[25,26] Further, it was established in a series of studies on the animal models of Alzheimer's disease[27–30] that

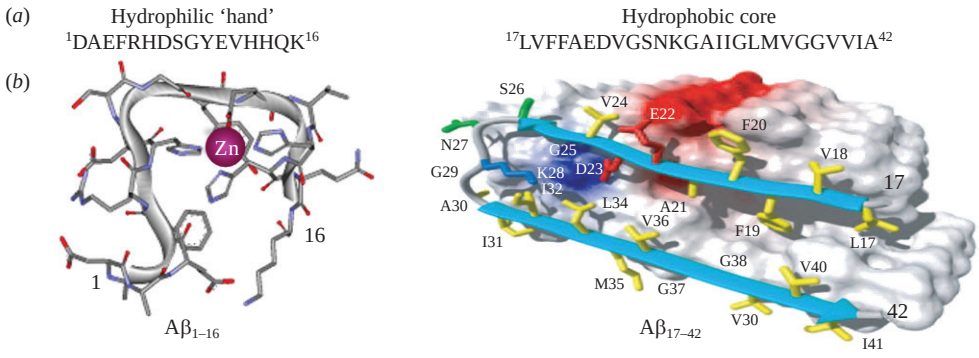


Figure 1 (*a*) Primary structure of $Aβ_{1–42}$ and (*b*) representation of $Aβ_{1–16}$ and $Aβ_{17–42}$ three-dimensional structures. On the left is the NMR solution structure of Aβ metal binding domain in complex with Zn^{2+} [Protein Data Bank (PDB) code 1ZE9].[43] On the right is the structure of β-sheet formed by the residues 17–42 of Aβ, obtained by analysing the structure of $Aβ_{1–42}$ fibrils.[16]

the molecular agent causing formation of pathologic amyloid plaques in the brain tissue is a conformationally or chemically modified version of Aβ.[31–33] However, the precise molecular nature of this agent remains unknown.[34] Different isoforms of Aβ from plaques including, for example, the version phosphorylated at serine 8 or containing pyroglutamic acid at position 3[35,36] as well as noncovalent complexes of Aβ with other biomolecules[7] were considered as putative pathogenic agents of Alzheimer's disease. We have suggested that the chemically modified isoforms of Aβ, which significantly affect the processes of its interaction with zinc ions,[37] should be of particular interest as potential drug targets and/or biomarkers in the diagnostic and therapy of AD.[38,39]

Interaction of zinc ions with Aβ

Initially it was established using model peptides that the segment 1–16 was the zinc-binding domain of Aβ.[13,40–42] Three-dimensional structures of this domain in the Zn^{2+}-loaded and Zn^{2+}-free states have been solved by NMR.[43] In the Zn^{2+}–$Aβ_{1–16}$ complex, zinc is tetrahedrally coordinated to His6, His13, and His14 through their N-δ1, N-ε2, and N-δ1 atoms, respectively, and to Glu11 through its O-δ atom. The molecular mechanism of interaction of zinc ions with Aβ includes two stages.[44] In the first stage a zinc ion binds with side chains of the residues Glu11, His13, and His14 in the pre-structured segment 10–15 of the domain (Figure 2). In the second stage the side chain of residue His6 enters zinc ion coordination sphere, resulting in an ordered compact structure of the whole domain 1–16 in complex with single zinc ion. We have determined that the segment 11–14 (Glu-Val-His-His) of Aβ acts not only as the primary recognition site for zinc ions,[43,44] but also controls the processes of zinc-induced Aβ oligomerization.[14,45,46] Earlier, it was found that the coordination of Zn^{2+} by His13 is critical for zinc-induced aggregation of human Aβ.[47] It is noteworthy to mention that the three-dimensional structure of segment 11–14 is rigid and remains practically unchanged both in the intact Aβ and in the Aβ complex with zinc ion.[43,48] We have shown that secondary structure of the segment 11–14 is formed by the left-handed polyproline-II type helix.[49] The polyproline-II segments are known to have high propensity to participate in protein–protein interactions.[50] Taken together these characteristics (participation in zinc binding, above average structural stability, and propensity to be involved in biomolecular interactions) allow one to consider segment 11–14 as the structural and functional determinant of Aβ.

One could rationally argue that amino acid changes in the site 11–14 perturb the conformation of not only this site but of the whole domain as well as crucially influence Aβ interactions with zinc ions. In support of this suggestion, the following should be considered: as opposed to other mammals, rats and mice are invulnerable to AD.[51,52] A key factor in such resistance could be the three amino acid substitutions (Arg5Glu, Tyr10Phe, and His13Arg) in the metal-binding domain, which represent the only discrepancy between human and rodent Aβ sequences. Indeed, our studies of the interactions of the rat Aβ metal-binding

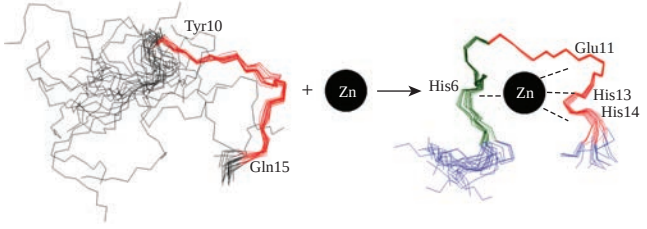


Figure 2 Schematic representation of $Aβ_{1–16}$ interaction with Zn^{2+}. Three-dimensional structure of $Aβ_{1–16}$ in the absence (left part, PDB code 1ZE7) and in the presence (right part, PDB code 1ZE9) of Zn^{2+}. Reproduced with changes from ref. 43.

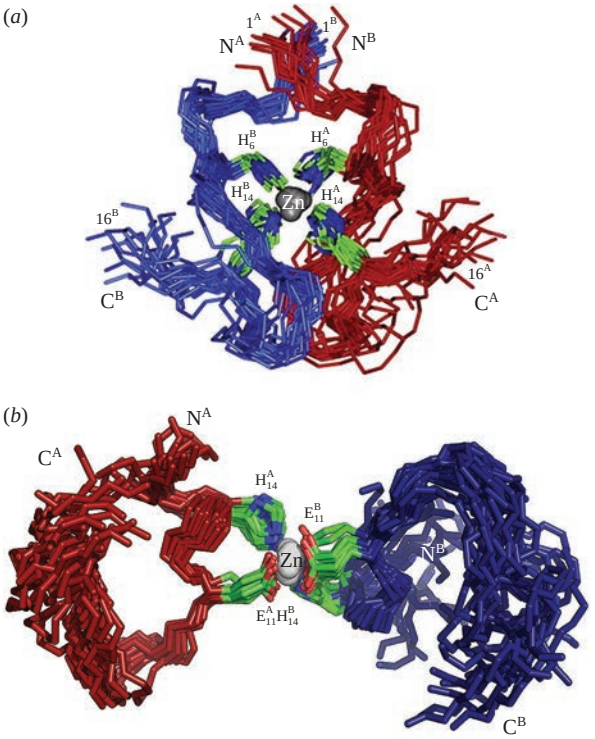


Figure 3 Structures of (*a*) rat and (*b*) human $Aβ_{1–16}$ dimers complexed with Zn^{2+} in solution. For each complex the family of 20 calculated NMR structures is presented (Protein Data Bank codes 2LI9 and 2MGT for rat and human $Aβ_{1–16}$, respectively). Only the backbone atoms (Cα, C, and N) and the side chains of the His residues are shown. The N- and C-termini of both chains are labeled. Structure of the rat dimer was reproduced from ref. 53 with permission from Elsevier.

domain with a zinc ion show clear differences between the rat and human domains in the set of residues that coordinate zinc ion, and in the structural organization of the zinc–peptide complex under close-to-physiological conditions[53,54] (Figure 3). All these differences define resistance of rats to AD, thus supporting the crucial role of zinc-mediated conformational changes of the metal-binding domain in the pathology.

We have recently established the molecular mechanism of zinc-induced oligomerization of the metal-binding domain of human Aβ.[55] Oligomerization starts with formation of zinc–peptide monomeric complexes, and subsequently proceeds *via* dimerization of these complexes, where zinc ion is coordinated by the side chains of residues Glu11 and His14 from the interacting peptide molecules (Figure 4). Afterwards, conformational rearrangement in the segments 6–14 of each subunit leads to formation of the second zinc-dependent dimerization interface composed of residues His6 and His13. The dimer becomes a seed of further zinc-dependent oligomerization leading to formation of higher order soluble oligomers that are transformed into insoluble aggregates. In contrast to the earlier concepts of polymorphism of the Aβ metal-binding domain within zinc-dependent Aβ oligomers,[15] our data demonstrate that this domain in such oligomers has a distinct three-dimensional structure.[55] This structure of the domain makes it possible for each Aβ molecule to interact with two other Aβ molecules through zinc ions. This triggers a 'chain reaction' of zinc-induced Aβ oligomerization. Our results also indicate that the extent of conformational freedom of residue His6 determines the propensity of a $Aβ_{1–16}$ isoform to undergo zinc-induced oligomerization. A particularly important role in this potentially pathogenic process is played by the isomerization of Asp7 (see below). This chemical modification greatly enhances the ability of the metal-binding domain 1–16 Aβ to undergo zinc-dependent oligomerization due to intramolecular conformational changes, which sterically facilitate the possibility

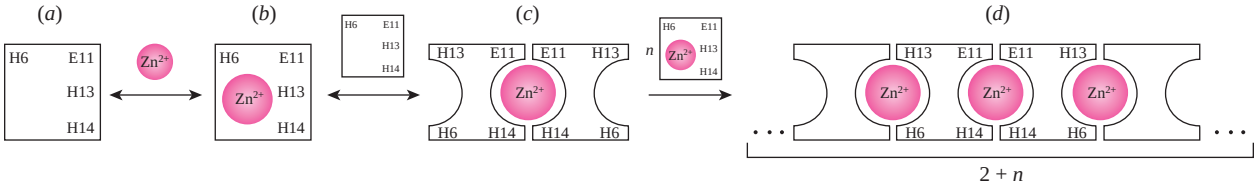


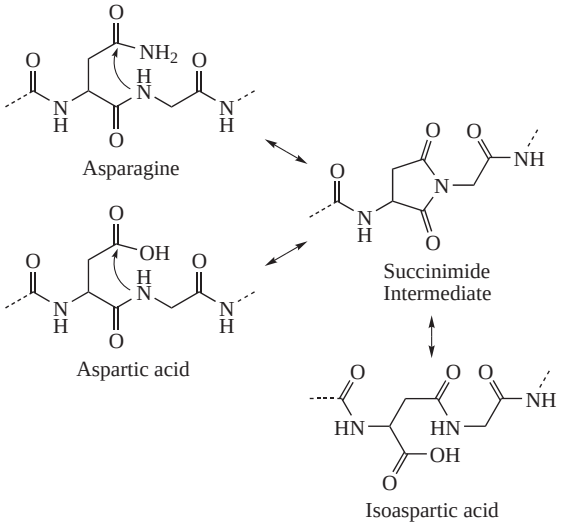
Figure 4 Schematic representation of zinc-induced oligomerization of the Aβ metal binding domain. Stages of oligomerization are shown: (*a*) peptide in free state, (*b*) peptide in complex with Zn^{2+}, (*c*) zinc-induced peptide dimer, and (*d*) zinc-induced peptide oligomer formed around the dimer.

for the 11–14 site to interact with other Aβ molecules.[37,56] Zinc-induced oligomerization of the intact Aβ could be triggered due to the formation of zinc-dependent heterodimers between intact and isomerized Aβ molecules through segments 11–14 of the interacting subunits.[56] Therefore, directional blocking of this segment should significantly slow down or stop the zinc-induced Aβ oligomerization and as a consequence, the development of cerebral β-amyloidogenesis.

Effect of Asp7 isomerization on the structural and functional characteristics of Aβ *in vitro*

Isomerization of aspartate and asparagine residues, resulting in the formation of isoaspartate (isoAsp, β-Asp) is a widespread chemical modification of damaged and/or long-living proteins both *in vitro* and *in vivo*[57] (Scheme 1). The isomerization process occurs in peptides and proteins spontaneously with time and appears to be a consequence of the degradation process during aging.[58] The isoaspartate, β-Asp, differs from the normal aspartate, α-Asp, structurally, but has the same molecular weight. The transformation of normal Asp or Asn residues into isoAsp occurs *via* a cyclic succinimide stage through a nonenzymatic intramolecular rearrangement of the peptide bond following the residue.[58] As a result, the main chain of the peptide backbone is lengthened by one CH_2 group, and, correspondingly, the side chain is shortened by the same group, thus leading to substantial changes in the structure of the transformed protein. Usually, after the isomerization of aspartate residues the protein loses its normal function and structure and is degraded. *In vivo* there is a special enzymatic system which returns β-aspartate into its normal α-Asp form, but this system works only inside the cell and cannot repair extracellular proteins.[59] Thus, extracellular isoAsp-containing proteins may be accumulated in the organism, such as it happens in Alzheimer's disease.[60,61]

We have established that isomerization of the aspartate residue in the 7-position crucially influences such processes as zinc and copper ion chelation by the metal-binding domain of Aβ,[43,62] zinc-dependent oligomerization of Aβ,[37] and hydrolysis of Aβ by the angiotensin converting enzyme.[63] Since each of these molecular processes is closely related to the aggregation ability of Aβ and thus plays a potentially crucial role in AD progression, we have suggested that the Aβ isoform carrying isoAsp7 residue (isoAβ) can represent a candidate pathogenic agent of AD.[38]



Scheme 1 Conversion of asparagine and aspartic acid to isoaspartic acid through succinimide intermediate.

Isomerization of Asp7 enhances neurotoxicity of Aβ

It is suggested that pathogenicity of amyloid peptides is associated with their cytotoxic properties. We investigated toxic effect of $isoA\beta_{1–42}$ compared to the intact $A\beta_{1–42}$ on human neural stem cells NSC-hTERT, undergoing differentiation,[64] and on the human neuroblastoma cell lines SK-N-SH[64,65] and SH-SY5Y.[66] In all cases the toxic effect of $isoA\beta_{1–42}$ was substantially stronger than that of $A\beta_{1–42}$. For example, incubation of cells with 15 μM of $isoA\beta_{1–42}$ resulted in death of almost two-thirds of the cells, while in the presence of the same amount of $A\beta_{1–42}$ only one-third of cells died.[67] Neuronal cell death when exposed to amyloid peptides may be related to the altering of intracellular redox regulation. Indeed, the cytotoxic effect of $isoA\beta_{1–42}$ and $A\beta_{1–42}$ is accompanied by increased level of reactive oxygen species and decreased level of intracellular glutathione.[64,65,67,68] However, these effects for both peptides are similar and do not explain the difference in their toxicity. We have shown[66] that, in contrast to $A\beta_{1–42}$, the redox determinant of $isoA\beta_{1–42}$ toxic effect on SH-SY5Y cells is represented by a significant reduction in the level of intracellular NO. Also it was shown on neuroblastoma cells SK-N-SH and THP-1 monocytes that $isoA\beta_{1–42}$ significantly stronger than $A\beta_{1–42}$ activates production of tumor necrosis factor TNFα, which is a stimulator of cell death.[69] The $isoA\beta_{1–42}$ toxic effect on neuronal cells is not only more pronounced but also more specific than that of $A\beta_{1–42}$, since the effect of $isoA\beta_{1–42}$ is caused to a large extent by the induction of apoptosis, whereas in the case of $A\beta_{1–42}$ cell death by necrosis is more typical.[67] It was shown that the $isoA\beta_{1–42}$ toxic effect is associated with activation of a number of intracellular apoptotic signaling pathways.[64] Thus, in SK-N-SH cells $isoA\beta_{1–42}$ causes activation of the pathways mediated by phosphoinositide-3-kinase (PI3K), phospholipase C (PLC), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38MAPK), and c-Jun N-terminal kinase (JNK).[64] Human $isoA\beta_{1–42}$ does not have a toxic effect on the primary culture of cells derived from the cerebral cortex of rat embryos.[70] Under the same conditions $A\beta_{1–42}$ caused the death of these cells. It can be assumed that $A\beta_{1–42}$ and $isoA\beta_{1–42}$ induce cell death through binding to different specific components on their surface, and absence of components specific to the human $isoA\beta_{1–42}$ on the surface of rat neuronal cells determines their resistance to the peptide. It is known that $A\beta_{1–42}$ interacts with acetylcholine receptors on the surface of neuronal cells.[71] We have created models of the interaction of metal-binding domains of the amyloid peptide $A\beta_{1–16}$ and $isoA\beta_{1–16}$, with extracellular domains of the neuronal nicotinic acetylcholine receptors consisting of α7 subunit pentamers (7nAChR). It has been found that the character of interaction of the intact and isomerized peptides with the receptor differ significantly. $A\beta_{1–16}$

binds to the receptor both within the channel, and on the receptor outer surface in the area of interface between the α7 subunits, while isoA$\beta_{1–16}$ binds only to the external surface of the receptor. Modelling results have been confirmed by our data on inhibition of the 7nAChR on the surface of SH-SY5Y cells by bungarotoxin, a specific inhibitor of these receptors. Inhibition of the receptor resulted in a loss of sensitivity of cells to the A$\beta_{1–42}$ toxic effect, but did not affect the toxicity of isoA$\beta_{1–42}$. Thus, 7nAChR is involved in the mechanism of A$\beta_{1–42}$ toxicity but not of the isoA$\beta_{1–42}$.

IsoAβ induces cerebral β-amyloidogenesis when introduced in transgenic mice models of Alzheimer's disease

A key feature of the AD pathogenesis is cerebral β-amyloidogenesis, a process of forming amyloid plaques in the patient's brain. Cerebral β-amyloidogenesis in animal models is greatly accelerated when they are injected with preparations of homogenized brain of the patients with AD.[72] IsoAβ constitutes a significant part of all Aβ molecules present in the AD brain extracts[60,73] and also can be spontaneously formed in the synthetic Aβ peptide preparations.[43,57] By analogy with the earlier studies[34,74] it can be suggested that isoAβ acts as aggregation seed and/or corruptive template compelling the physiological pool of endogenous Aβ to be converted into oligomers and consequently into aggregates.

We have tested the ability of synthetic isoA$\beta_{1–42}$ to induce cerebral β-amyloidogenesis in susceptible transgenic mouse model of AD.[75] We have used APP/PS1 doubly tg mice which manifest characteristic cognitive features of AD-like pathology and possess significant amounts of dense-core congophilic amyloid plaques starting from 4 to 6 month age.[76,77] The experimental groups included male animals which were grown under specific pathogen-free conditions and were subjected to intravenous injections of isoA$\beta_{1–42}$ starting from 2 month age. After serial (at monthly intervals) inoculations with the peptide the host mice were sacrificed, the brains were extracted and sagital brain sections prepared by cryotomy were analyzed histochemically using Congo Red staining (Figure 5).

The congophilic deposits visualized in the brains of all tg animals were similar in terms of their localization and size distribution in the brain parenchyma. However, the quantification of the amyloid plaques has revealed much more robust cerebral β-amyloidogenesis in the isoAβ-inoculated 4- and 10-month-old APP/PS1 tg mice compared to untreated littermate controls, whereas intravenous inoculations with PBS or synthetic peptide A$\beta_{1–42}$ were ineffective (Figure 5). Intravenous injection of isoAβ into wild-type mice did not induce any amyloid plaque formation in the animals regardless of their age. That is in line with the previously published data concerning the prerequisite role of endogenously produced Aβ for *in vivo* seeding.[33] Thus, using the transgenic model of AD we have identified the peripherally applied isoA$\beta_{1–42}$ as an inductive agent of the amyloid plaque formation *in vivo*.[75]

As described in the above sections, there is increasing evidence in favor of important role of the metal-binding domain (A$\beta_{1–16}$) in aggregation of the full-size Aβ molecule, since zinc-induced dimerization occurs in the 1–16 region and subsequently drives all the molecules to form oligomers,[15,53,55,56] while the A$\beta_{17–42}$ peptide is known to form nonfibrillar diffuse deposits, which are not associated with AD.[78] A$\beta_{1–16}$ constitutes an independent folding unit in the full-length Aβ[13,16,43,48] and is also found as autonomous APP-derived species in cerebrospinal fluid.[79] Using 5XFAD transgenic mice as a model of AD, we have shown that a single intracerebral injection of 5 μg of synthetic isoA$\beta_{1–16}$ peptide results in significant acceleration of cerebral β-amyloidogenesis, while the injection of A$\beta_{1–16}$ did not.[80] Thus, essentially

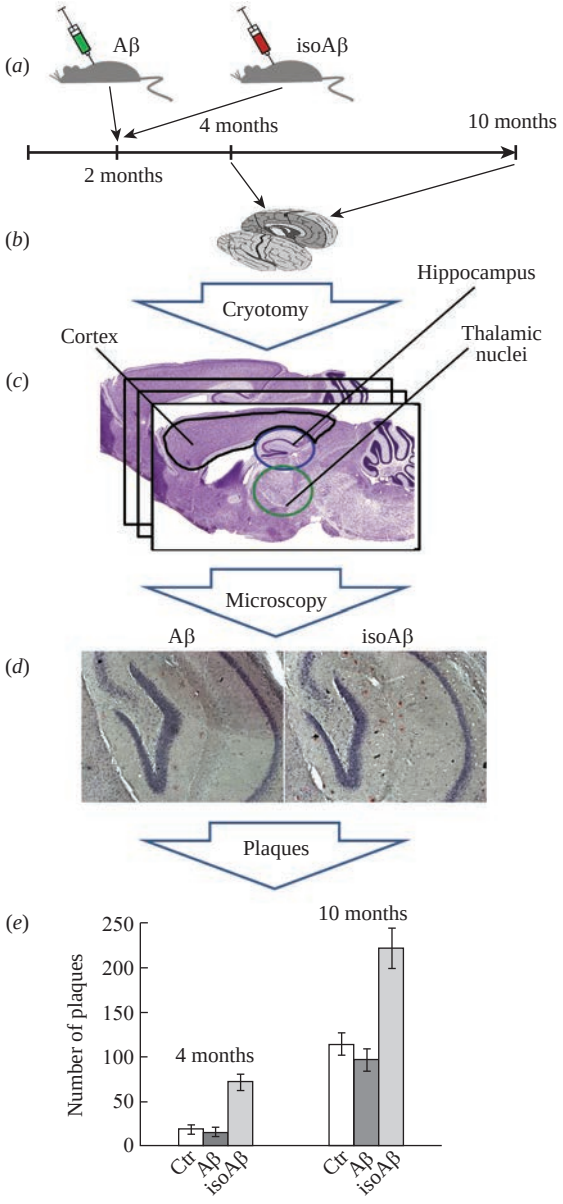


Figure 5 Induction of congophilic amyloid plaques formation in the brain of B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J transgenic mice. (*a*) Mice received two intravenous injections of A$\beta_{1–42}$ or isoA$\beta_{1–42}$ solution with 1 month intervals between injections starting from 2 month age. (*b*) The host mice were sacrificed at the age of 4 and 10 months, the brains were extracted and (*c*) sagittal brain sections (25 lm-thick) prepared by cryotomy were (*d*) analyzed histochemically using Congo Red staining. (*e*) Number of plaques was counted in cortex, hippocampus, and thalamic nuclei; Ctr – control, Aβ and isoAβ – mice received A$\beta_{1–42}$, and isoA$\beta_{1–42}$, respectively.

similar results have been obtained: (1) applying different methods of administering the compounds, (2) in two different transgenic mice models of AD, and (3) using Aβ peptides of various length that included the isoA$\beta_{1–16}$ as the common unit. This demonstrates that the isoA$\beta_{1–16}$ segment plays a key role in the amyloidogenic effect of isoA$\beta_{1–42}$. Moreover, A$\beta_{1–16}$ with isomerized Asp7 acts as the minimal currently known Aβ aggregation seed. Reduction of the amyloidogenic agent of AD from isoA$\beta_{1–42}$[75] to isoA$\beta_{1–16}$[80] not only has the fundamental aspect linking pathogenic property to a much smaller object, but also provides the rationale for focusing on the metal-binding domain as a prospective drug target to control the AD β-amyloidogenesis.

A mass spectrometric method for quantitative determination of the relative abundance of synthetic peptides with isomerized aspartate residues in the 7-position, corresponding to the segment 1–16 of Aβ, was developed by us using relative intensities of

diagnostic ions characteristic of this isoform in MS/MS spectra.[81] This method can be used for quantitative analysis of the abundance of the isomerized form in binary mixtures at low peptide concentrations. Though this approach was developed on a model system, it appears to be adequate to be further used to get a reliable estimate of the level of isoAβ in human blood plasma samples, thus allowing the method to be employed in further clinical trials to validate the role of the isoAsp7-containing Aβ species as biomarkers of AD.

Tetrapeptide fragment of the acetylcholine receptor that is ion-complementary to segment 11–14 of Aβ as a potential anti-amyloid drug

Given that zinc-induced oligomerization of intact Aβ can be initiated due to the formation of zinc-dependent heterodimers from the intact and isomerized Aβ molecules through segments 11–14 (Glu11-Val12-His13-His14) of the interacting subunits,[56] directed blocking of this site should significantly slow down or stop zinc-induced Aβ oligomerization and, consequently, the development of cerebral β-amyloidogenesis. Antibodies, small molecules, peptides, peptidomimetics and similar substances can be employed as candidate molecules capable of specific binding to the site 11–14 of Aβ, thus disrupting zinc-dependent aggregation of Aβ. Passive immunotherapy is widely used in modern medicine; it has been viewed for a long time as a prospective approach of the anti-amyloid strategy in AD therapy. Currently there is a number of antibodies (bapineuzumab, gantenerumab, aducanumab), which when binding to Aβ block the 11–14 site from interactions, and thus prevent Aβ aggregation and break up the already present amyloid plaques.[82] However, clinical trials showed unacceptable toxicity of these antibodies, which is most likely associated with increased neuronal dysfunction.[83]

It is known that Aβ interacts with acetylcholine receptors, and it has been shown recently that the 11–14 segment is critical for these interactions, however a corresponding site in the receptor has not been identified.[84] We have found using bioinformatic approaches that acetylcholine receptor (α4 subunit) carries the tetrapeptide segment His-Ala-Glu-Glu (HAEE) which is ion-complimentary to the 11–14 segment of Aβ. This prompted us to suggest a possible role of the HAEE segment as a partner for Aβ.[85] Experimental tests *in vitro* revealed that the synthetic peptide [Acetyl]-His-Ala-Glu-Glu-[Amide] (Ac-HAEE-NH_2) specifically binds to the metal-binding domain of 1–16 Aβ at the 11–14 site, blocks zinc-induced dimerization and sharply slows down aggregation of the full-length Aβ.[85]

Intravenous injection (*in vivo*) of the Ac-HAEE-NH_2 peptide dramatically decreases the development of cerebral β-amyloidogenesis in AβPP/PS1 mice, used as a recognized animal model of AD. The average number of amyloid plaques in the brain section was reduced from 14.2±3.1 (control animals) to 5.8±2.1 (for animals subjected to therapy).[85] By this indicator the effectiveness of Ac-HAEE-NH_2 greatly exceeds the anti-amyloid effect of Alzhemed (Tramiprosate), one of the most well known anti-aggregation drug candidates for treatment of AD[86] – when using Alzhemed in transgenic mice TgCRND8, decrease in number of amyloid plaques constituted 30% compared to control animals.[87] Based on the data pertaining to the molecular mechanism and functional effectiveness of Ac-HAEE-NH_2 this peptide, which blocks the primary zinc binding site 11–14 of Aβ, represents an important drug candidate aimed at suppressing cerebral β-amyloidogenesis in AD.

Conclusions and perspectives

Studies of the molecular mechanism of zinc ions interaction with Aβ and its natural isoforms showed a critical impact of the Asp7 isomerization on the ability of Aβ to undergo zinc-induced oligomerization and involve in this oligomerization the endogenous Aβ molecules. This modification also leads to a sharp increase in the neurotoxicity and aggregation propensity of Aβ. Thus, isoA$\beta_{1–42}$ and isoA$\beta_{1–16}$ constitute potentially pathogenic agents of Alzheimer's disease, making it possible to use appropriate Aβ variants as biomarkers for early diagnostic of AD, and drug targets in the therapy of this pathology.

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