

## The Convergence of Alzheimer's Disease Pathogenesis Concepts

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**Abstract**—Advances in the research of molecular factors involved in the onset and progression of Alzheimer's disease, have led to the creation of several pathogenesis concepts of the most common neurodegenerative disease in the world, and amyloid, cholinergic, and neuroinflammatory hypotheses became leading. Over past twenty years, based on these hypotheses, hundreds of drug prototypes were developed, but none of them were able to stop the development of Alzheimer's disease. In this review, based on the latest experimental data on structure-function properties of chemically modified amyloid-beta isoforms, the concept of the origin and the mechanism of action of amyloid-beta with isomerized Asp7 residue, as a molecular agent of Alzheimer's disease pathogenesis, is presented. This concept makes it possible not only to combine the most important aspects of existing hypotheses but also indicates ways of creating agents for fighting Alzheimer's disease with a principally new mechanism of action, "disease-modifying drugs."

**Keywords:** Alzheimer's disease, amyloid-beta, isoaspartate, cerebral amyloidogenesis, amyloid plaques, neuroinflammation, neurodegeneration,  $\alpha 4\beta 2$  subtype nicotinic acetylcholine receptor

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Alzheimer's disease (AD) is today's most widespread neurodegenerative proteinopathy [1], which affects more than 44 million people [2]. Clinical manifestations of AD are expressed in inevitable cognitive decline and accompanied by organic brain destruction, which, in the end, results in patient death via respiratory arrest. Hereditary variants make less than 1% of total AD cases and are associated with mutations [3, 4], resulting in constitutional excess above the physiologically normal level of amyloid-beta (A $\beta$ ), a short polypeptide of 39–43 amino-acid residues length with a heterogeneous C-terminal site [5]. Causes of the onset of sporadic variants, which make over 95% of all AD cases, remain unknown, but they are closely associated with abnormal aggregation of endogenous A $\beta$ . There exist three main neuromorphological signs, which unambiguously confirm (postmortem) the diagnosis of all AD cases: (1) the presence, in certain brain regions, of typical extracellular aggregates (amyloid plaques), whose main components are various A $\beta$  isoforms and biometal ions (zinc, as well as iron and copper); (2) the formation of intracellular neurofibrillary tangles (whose main component is hyperphosphorylated tau protein); and (3) neuronal degeneration [6].

Extensive experimental data support a contemporary version of the amyloid cascade hypothesis of AD pathogenesis [7]. This hypothesis states that slow steady accumulation of A $\beta$  aggregates in the brain (cerebral amyloidogenesis, CA) initiates AD develop-

ment by triggering a complex signaling cascade, which accelerates the pathological transformation of tau and results in neurodegeneration and clinical dementia. Such factors as familial mutations, traumatic brain injuries, specifics of lifestyle and environment, inflammation processes, and cardiovascular diseases increase the risk of the disease or influence pathological processes [8].

Endogenous A $\beta$  is formed as a result of proteolysis of the amyloid protein precursor [9]; it is present in brain tissues and in peripheral organs over a lifetime. The A $\beta$  level in blood and cerebrospinal fluid varies depending on age and individual specifics of patients, hence it cannot serve as an AD biomarker [10]. The physiological role of A $\beta$  can be the regulation of a synaptic function, protection from infections, repairing damaged sites of the blood-brain barrier, and compensation of trauma consequences [11]. A $\beta$  concentration in cerebrospinal fluid is 5 to 15 times higher than in plasma. It is not clear how A $\beta$  is removed from blood, however it is established that about 60% A $\beta$  goes from the brain into the peripheral circulatory system [12]. At the same time, A $\beta$  molecules circulating in blood, which are mainly generated by platelets, can get into the brain at system circulation disturbance and significantly accelerate AD development [10].

According to the neuroinflammation hypothesis, disturbance in the regulation of the central nervous system immune response is considered a key factor of AD etiology, and neuroinflammation is regarded as an

integral mechanism of AD pathogenesis [13]. The persistent anti-inflammatory response in the brain of AD patients was earlier considered a reaction to numerous neuronal loss. However, at present, the persistent immune response in the brain is not only linked to neurodegeneration but also considered a driving force of pathological processes involving A $\beta$  and tau [14]. It is shown that peripheral inflammation and abnormalities of peripheral immune response in AD are associated with oxidative stress increase in the peripheral circulatory system and in the brain [15]. An increase in oxidative stress is described in peripheral blood lymphocytes obtained from AD patients. Neuroinflammatory processes in the brain are mediated mainly via the innate immune system, which includes astrocytes and microglial cells, as well as cytokines, chemokines, and growth factors. At the same time, the central nervous system is accessible for lymphocytes and monocytes, circulating in blood, which indicates an intensive interaction between immune and central nervous systems, aimed at maintaining homeostasis [16]. A $\beta$  molecules, generated by platelets, may act as mediators of this interaction, and, at A $\beta$  metabolism disturbance in the brain, may be a source for the formation and distribution of amyloid plaques in AD [17].

Besides A $\beta$  and its chemically modified isoforms, amyloid plaques contain many other components, including various proteoglycans and glycosaminoglycans, carbohydrate-binding proteins of the innate immunity system (in amyloid plaques, serum amyloid protein P (SAP) is represented the most), nucleic acids, biometal ions, lipids, and transport proteins. It is accepted that these cofactors may seriously affect A $\beta$  aggregating processes [18]. However, by now, only for zinc ions, the absolute necessity for amyloid plaque formation in model animals is experimentally proven [19]. Zinc, released into glutamatergic synapse due to ZnT3 transporter activity, is vital for the maintenance of memory and cognitive functions [20]. ZnT3 is expressed only in damaged via amyloid pathology grey matter areas. Zinc, released into glutamatergic synapse through ZnT3, causes amyloid pathology in transgenic mice [21, 22], which explains the abnormally high content of zinc ions in amyloid plaques [23, 24]. Very low in physiological conditions, extracellular concentration of zinc ions (<1  $\mu$ M) in neuronal activity can run up to 100–300  $\mu$ M in synaptic clefts of glutamatergic synapses [25]. It is important to notice that zinc ions also promote the formation of highly toxic zinc-bound A $\beta$  oligomers [26].

Cholinergic synapses are spread everywhere in the human central nervous system, and the studying of synaptic neurotransmission in norm and AD led to the creation of the cholinergic hypothesis, which associates the progressive loss of limbic and neocortical cholinergic innervation with cognitive decline and neurodegeneration [27]. The following facts underlay the cholinergic hypothesis: (1) the detection of presynaptic cholinergic marker depletion in the brain cortex in

AD; (2) the discovery that nucleus basalis of Meynert in the basal forebrain is a source of cortical cholinergic innervation, which is subjected to grave neurodegeneration in AD; (3) cholinergic antagonists impair memory, while agonists exert the opposite effect. The cholinergic hypothesis was practically implied in the development and successful application of cholinesterase inhibitors as a means of symptomatic treatment for AD patients. Five anti-AD drugs, approved by the US Food and Drug Administration, are used worldwide and include four cholinesterase inhibitors (Tacrine, Donepezil, Rivastigmine, Galantamine) [28].

It is suggested that amyloid plaques cause the degeneration of cholinergic fiber endings [29]; at the same time, at early stages of AD, first of all occurs the impairment of nicotine cholinceptors (nAChR) of  $\alpha$ 4 $\beta$ 2 subtype ( $\alpha$ 4 $\beta$ 2-nAChR) [30], with which A $\beta$  forms specific complexes [31, 32].  $\alpha$ 4 $\beta$ 2-nAChR receptors are widespread in the brain cortex. The loss of cognitive functions via middle-stage AD patients is accompanied by a substantial reduction in availability (according to data of positron emission tomography) of precisely  $\alpha$ 4 $\beta$ 2-nAChR [33]. The 11-Glu-Val-His-His-14 (11EVHH14) site of A $\beta$  is critical for interactions between A $\beta$  and nAChR [34]. With the help of bioinformatic approaches, it is established that the extracellular N-terminal domain of the  $\alpha$ 4 $\beta$ 2-nAChR  $\alpha$ 4 subunit includes 35HAEE38—the 35-His-Ala-Glu-Glu-38 fragment (conservative in human, mouse, and chicken), which is ionic-complementary to the 11EVHH14 A $\beta$  site. An experimental test using an optical biosensor on a surface plasmon resonance effect showed that a synthetic [Acetyl]-His-Ala-Glu-Glu-[Amide] (Ac-HAEE-NH<sub>2</sub>) analogue of the 35HAEE38 site is specifically bound to the 11EVHH14 site of the immobilized metal-binding 1–16 A $\beta$  domain [35]. Thus, we suggest that the interaction interface of A $\beta$  and  $\alpha$ 4 $\beta$ 2-nAChR is formed via 11EVHH14 and 35HAEE38 sites of these molecules, correspondingly.

The ability of A $\beta$  aggregates, extracted postmortem from AD patient brains, inducing AD development was for the first time demonstrated on primates, to whom the proper autopsy material was administered intracerebrally [36, 37]. Further, in a series of studies on animal AD models [38–41], it was established that a molecular agent causing the formation of pathological amyloid plaques in brain tissues constitutes a conformational or chemically modified variant of A $\beta$  [42–44]. It was also shown that, in CA induction, amyloid plaques are formed in model animals in a day, then, after one to two days, the activation of microglial cells and their migration to the plaques are observed, which is accompanied by local alterations in axons and dendrites of adjacent neurons [45]. The spreading of amyloid plaques through brain structures occurs extracellularly [46]. As a result of incubation in hippocampus splice culture, synthetic A $\beta$  becomes a pathological agent causing CA via seeding mechanism (at

presence of a minor amount of brain homogenate from AD patients) [47]. The seeding CA initiation mechanism in AD suggests a significant alteration of the native structure of endogenous A $\beta$  under the influence of interaction with pathological A $\beta$  molecules [48], present in amyloid plaques. This mechanism is confirmed by the fact that minor amounts of pathologically changed A $\beta$  molecules, contained in material from AD patients, caused the transition of native A $\beta$  molecules into a pathological state [49, 50].

The tendency of full-size A $\beta$ s to spontaneous aggregation prevents determination of their spatial structures with modern biophysical methods [51]. However, the N-terminal 1–16 (A $\beta$ 16) fragment, which is also present in the human organism as an independent factor [52], is an autonomous metal-binding A $\beta$  domain, stable *in vitro* [53]. The structure of this domain is successfully determined in a free state and in complex with zinc ion in physiologically relevant conditions [54]. It turned out that rigid spatial organization of the main polypeptide chain of the 11EVHH14 site remains practically unchanged in intact A $\beta$ 16 and in the A $\beta$ 16 complex with zinc ion [54, 55]. The secondary structure of the 11EVHH14 site constitutes a left-handed helix of the polyprolin II type [56], possessing high predisposition to participate in protein-protein interactions [57].

The molecular mechanism of interaction between zinc ions and A $\beta$ 16 is established. First, zinc ion is chelated by Glu11, His13, and His14 side chains, then takes place the folding of N-terminal 1–8 A $\beta$ 16 site with the following formation of additional coordination linkage between zinc ion and His6 side chain, resulting in the appearance of a well-ordered compact structure of the whole 1–16 domain [58]. It is also shown that the 11EVHH14 site of amyloid-beta acts not only as primary center of zinc ion recognition but also controls zinc-induced A $\beta$  dimerization processes [59, 60].

Complexes of the zinc ion with a metal-binding A $\beta$  domain may constitute either compactly folded monomers, where zinc ion is coordinated via four chelator (His6, Glu11, His13, and His14) of one A $\beta$ 16 molecule, or structurally unfolded dimers, where one zinc ion and Glu11 and His14 amino acid residues of interacting subunits form an intermolecular interface. In the presence of zinc ions, the metal-binding domain within full-size forms of A $\beta$  is mainly unfolded and is involved in intermolecular bonds that stabilize polymorphic oligomers and aggregates of A $\beta$  [61]. The molecular mechanism of zinc-dependent A $\beta$ 16 oligomerization includes the formation of unfolded dimer, then in each subunit of the dimer occurs the regrouping of side chains His6 and His13, which lets these residues from two adjacent A $\beta$ 16 molecules form an additional molecular interface with one zinc ion. As a result, each A $\beta$ 16 subunit within oligomers has two intermolecular zinc-dependent inter-

faces [62]. Thus, the A $\beta$ 16-zinc-A $\beta$ 16 dimer is a seed of zinc-dependent oligomerization of A $\beta$ 16 molecules via “chain” mechanism. In forming oligomers, the conformation of the main chain of the 11EVHH14 fragment of each domain remains the same in free and in bound with zinc ion A $\beta$ 16 molecules [62]. By the total of all properties, 11EVHH14 site constitutes a structure-function determinant not only for A $\beta$ 16 in zinc-bound oligomers but, perhaps, also for full-size A $\beta$  molecules within amyloid plaques. Therefore, a new anti-amyloid strategy for AD therapy seems to be prospective, where substances, specifically binding to the 11EVHH14 site, will be used as medicinal agents preventing pathological A $\beta$  aggregation and the destruction of already formed amyloid plaques in AD [63].

The isomerization of Asp7 residue in A $\beta$  metal-binding domain plays an especially significant role in the interaction of A $\beta$  with zinc ions [64]. This chemical modification significantly increases the ability of A $\beta$ 16 to undergo zinc-dependent oligomerization due to intramolecular conformational changes, sterically facilitating the 11EVHH14 site ability to bind with other A $\beta$  molecules via zinc-dependent interfaces [65, 66]. In amyloid plaques, A $\beta$  with isomerized Asp7 residue (isoA $\beta$ ) is present in abnormally high amounts (about 50% of total A $\beta$  content) [67, 68]. IsoA $\beta$  is formed spontaneously as a result of “protein aging” [69] both of circulating and aggregated A $\beta$  molecules [51]. It is shown that isoA $\beta$  accumulation in brain tissues is a common process for elderly people; however, the isoA $\beta$  level is significantly higher in AD patients [70].

We made a suggestion that, under the action of inner or outer stress factors in the peripheral circulatory system (which seem to be more susceptible to different kinds of stress than the central nervous system), isoA $\beta$  is formed. Further, isoA $\beta$ , in interaction with the 35HAEE38 site of the extracellular N-terminal domain of the  $\alpha$ 4 subunit of  $\alpha$ 4 $\beta$ 2-nAChR, forms an abnormally long-lived complex, or “amyloid matrix,” while an intact A $\beta$  forms a relatively short-lived complex with the same site of the receptor, and, in such a complex, the normal function of A $\beta$  in the organism is fulfilled. On the contrary, the “amyloid matrix,” on the synaptic burst of zinc ion concentration, initiates zinc-dependent oligomerization of endogenous A $\beta$  molecules, as a consequence of which, in a short time, an amyloid plaque is formed on “amyloid matrix,” whose growth can be restricted by a microglial response. This extracellular plaque can exist for decades and does no direct harm to the neuron, since A $\beta$  molecules are not transported from it in significant amounts (because of their condensed state) into the cell and, correspondingly, no signal cascades are activated. Under favorable circumstances, amyloid plaques would be practically inert A $\beta$  deposits, at first insignificantly aggravating the life of neurons, but eventually causing their apoptosis (most likely, through the induction of intraneuronal tau protein phosphorylation [71, 72]), and, as a consequence, a

cognitive decline in the patient in the long term. However, if, for some reason, for instance, as a result of neuroinflammation, the necrosis of at least one neuron starts, then different cytosolic proteins will get into extracellular space, in particular, one of the most widespread cell proteins—glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is involved in AD pathogenesis [73], presumably, as a result of the formation of neurotoxic aggregates with A $\beta$  [74]. Upon reaching a certain critical number of amyloid plaques in brain tissues, the initial focus of neuronal necrosis may trigger a chain reaction of mass neuron loss, typical for late stages of AD. Thus, isoA $\beta$ , which is considered an end product of the pathological transformation of physiologically normal A $\beta$  undergoing various kinds of stress, can be a molecular agent of initiation of AD pathogenesis.

We tested this hypothesis on the animal model of AD. In 2013, it was for the first time shown that intravenous injection of synthetic isoA $\beta$  to transgenic mice of the B6C3-Tg(APP<sup>swe</sup>, PSEN1-dE9)85Dbo/j line results in abrupt acceleration of CA [75]. At the same time, there was no trace of amyloid plaques in wild-type control mice, which confirms the role of isoA $\beta$  as a seed of human endogenous A $\beta$  aggregation. Five years later, the possibility of the transition of a molecular agent, causing AD, from the peripheral system into the brain, was confirmed in independent studies using the parabiosis of the APP<sup>swe</sup>/PS1dE9 line transgenic mice and wild-type mice [76], and on transgenic mice of the APP<sup>swe</sup>/PS1dE9 line, to which were intravenously administered preparations of AD patients brain homogenates [77]. With the help of 5XFAD line transgenic mice, we established that the precisely metal-binding domain of isoA $\beta$  (isoA $\beta$ 16) is necessary and sufficient for exogenous induction of CA in animals [78]. Thus, using synthetic peptides isoA $\beta$  and isoA $\beta$ 16, we can arbitrarily turn on the molecular mechanism of CA in animal models of AD, where human A $\beta$  is constitutively expressed.

It is important to note that, according to this mechanism, amyloid plaques can be formed only on condition that endogenous A $\beta$  molecules with an amino acid sequence, identical to the human A $\beta$  sequence, are present in the organism. Three amino acid replacements (Arg5Gly, Tyr10Phe, and His13Arg) in the metal-binding domain A $\beta$  of rats and mice distinguish them from other mammals and are associated with resistance to Alzheimer type neurodegeneration in these rodents [79]. According to our hypothesis, precisely the His13Arg mutation completely prevents zinc-dependent oligomerization in these animals. A $\beta$  of the naked mole rat (*Heterocephalus glaber*) contains only this replacement, and this animal never has Alzheimer type neurodegeneration [80]. Earlier it was reported that, in the A $\beta$  of degu (*Octodon degu*), a single His13Arg replacement is also present, but that degu are allegedly susceptible to spontaneous Alzheimer

type neurodegeneration [81], however, later independent studies refute these data [82, 83].

Based on the critical role of “amyloid matrices,” i.e., complexes between isoA $\beta$  and  $\alpha$ 4 $\beta$ 2-nAChR, in the initiation of zinc-dependent formation of amyloid plaques in AD, we suggested that compounds, capable either to inhibit the “amyloid matrix” formation or hinder zinc-dependent A $\beta$  oligomerization on this matrix, can block CA and destroy amyloid plaques. A synthetic peptide [Acetyl]-His-Ala-Glu-Glu-[Amide] (Ac-HAEE-NH<sub>2</sub>)—N-acetylated and C-amidated analog the 35-His-Ala-Glu-Glu-38 (35HAEE38) site of the subunit  $\alpha$ 4 of nicotinic acetylcholine receptor  $\alpha$ 4 $\beta$ 2-nAChR—was tested as a substance for destroying amyloid matrices. Intravenous injection of Ac-HAEE-NH<sub>2</sub> to transgenic mice of the B6C3-Tg(APP<sup>swe</sup>, PSEN1-dE9)85Dbo/j line resulted in an abrupt decrease in the number of amyloid plaques in animal brains [84]. We have tested the ability of synthetic A $\beta$  and isoA $\beta$  analogs, phosphorylated at Ser8 residue, to inhibit zinc-dependent A $\beta$  oligomerization. The choice of these molecules is based on the influence of Ser8 residue phosphorylation on the blockage of zinc-dependent oligomerization of metal-binding A $\beta$  domain established earlier by us [60]. Intravenous injections of phosphorylated A $\beta$  and isoA $\beta$  resulted in significant retardation of CA in model animals [85, 86].

The following integral scenario of the CA molecular mechanism in AD is suggested: (1) because of unknown causes (most likely stress and aging), isoA $\beta$  appears in the brain; (2) the 11EVHH14 site of this chemically modified A $\beta$  isoform interacts with the 35HAEE38 site of the  $\alpha$ 4 subunit of  $\alpha$ 4 $\beta$ 2-nAChR, resulting in the “amyloid matrix”—a long-lived complex of isoA $\beta$  and  $\alpha$ 4 $\beta$ 2-nAChR—forming on the external surface of the neuron; (3) upon zinc ion concentration burst in the synaptic cleft, the intact A $\beta$  molecule “sticks” to the “amyloid matrix” via a zinc-dependent mechanism, herewith the 11EVHH14 fragment participates in the interface on behalf of this A $\beta$  molecule, while, on behalf of the “amyloid matrix,” the His6 and His13 residues of isoA $\beta$  molecules do so—and an already insoluble aggregate appears, at which on the external side an endogenous A $\beta$  molecule is located, however, already in the pathological conformation (as a result of interaction with isoA $\beta$  from “amyloid matrix”); (4) further, by the same pattern, endogenous A $\beta$  molecules, newly arrived from the extracellular space, “stick” on A $\beta$  molecules, aggregated on the initial “amyloid matrix,” and the growth of amyloid plaque goes on (to a certain canonic volume).

From this mechanism of amyloid plaque formation, it follows that the most effective and safe way to destroy this plaque would be to use structure analogs (peptide or peptidomimetics) of the 35HAEE38 site of the  $\alpha$ 4 subunit of  $\alpha$ 4 $\beta$ 2-nAChR, for example, an Ac-HAEE-NH<sub>2</sub> substance, which has a potential therapeutic effect already confirmed on an animal AD

model [84]. When interacting with an amyloid plaque, these analogs will progressively destroy intermolecular interfaces involving A $\beta$  fragments I1EVHH14 due to the specific binding with I1EVHH14 sites of aggregated A $\beta$  molecules. Herewith, the “amyloid matrix,” underlying this pathological pyramid eventually also will be destroyed [63], and the neuron that for many long years or even decades was burdened with amyloid plaques, will return to normal life.

The “amyloid matrices” concept, introduced in this work, is based on the convergence of leading hypotheses of AD pathogenesis and makes it possible not only to suggest an integral molecular mechanism of CA in this disease, but indicates the possible role of amyloid plaques in neurodegeneration in AD, and also the perspectives of developing disease modifying drugs, capable of effectively stopping the clinical course of the disease.

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