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Review article

Role of advanced glycation end products (AGEs) on the reactive oxygen species (ROS) generation in Alzheimer's disease amyloid plaque

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Abstract

In conditions of intensified generation or decreased breakdown, amyloid beta (Aβ) accumulates in the intersticium in the vicinity of synapses and originates cascades of biochemical events which make the Alzheimer's disease (AD) fundament. This Aβ accumulation connected with the expression of its precursor APP (amyloid precursor protein) on neuronal membranes in the synaptic region, and proteolysis of APP, induces the mentioned cascades which are intensively investigated. Marked APP proteolysis, connected with AD, induces Aβ generation, its aglomeration and aggregation, with the occurrence of amyloid plaques. Analyses of the plaque's structure indicate the presence of Aβ, metal ions and advanced glycation end products (AGEs). Near the plaques there are more cells, astrocytes and microglia. Passing through the monomer, dimer and trimer phase, Aβ aggregates in fibrils, which by glycation and connection with the supervene, or *in situ* formed AGEs, generate cross-β structures. During these events frequent contacts appear between Fe^{3+} on one monomer and MetS35 on the other, increasing Fe^{3+} reduction. According to recent investigations, this is the crucial component in the fundament of AD. MetS35 is a strong reducer. By the generation of redox-active Fe^{2+} which *in situ*, by Fenton reaction, generates non-toxic Fe^{3+}, hydoxyl radical and hydroxyl ion, the situation occurs in which hydroxyl radical attacks adjacent structures, especially lipid membranes. Lipid peroxidation occurs with consequent irreparable lesions. Fe^{3+} enters the redox cycle, and after that by the effect of Aβ again reduces into Fe^{2+}. The question is if AGEs, generated in the plaque, or arrived in it from the cytosol, have a direct impact on Fe^{3+} reduction, or if the effect is indirect through Aβ aggregation. In this respect the presented review attempts to give some actual knowledge about this problem.

***KEY WORDS*:** Alzheimer's disease, amyloid beta plaque, advanced glycation end products, ferric iron reduction

Introduction

A number of complex recent investigations about biochemical events during the evolution of amyloid plaque (SP, senile plaque) indicate a strong reductive amyloid beta (Aβ) capacity and its crucial role in the transformation (reduction) of non toxic redox-inactive Fe^{3+} (ferric iron) into toxic redox-active (ferrous iron, Fe^{2+}) (equation 1/Fe^{3+} reduction). The latter form (Fe^{2+}) loses its surplus of electrons (e^-), by oxidation (loss of electrons) through hydrogenii peroxydi in Fenton reaction, and transforms into redox-inactive Fe^{3+}, accompanied with the appearance of *OH (hydroxyl radical) and $^-$OH (hydroxyl ion). In its essence, this reaction is spontaneous, independent of catalysts, and depends exclusively on the quantity of indispensable substratum (equation 2/Fenton reaction) [1-8].

Senile plaques (SP), *i.e.* amyloid plaques, are typical characteristics of Alzheimer's disease (AD). Their occurrence in this disease in relation to the healthy population (persons without signs of AD), develops in certain regions of the brain, primarily in the hypocampus, amygdala, and cortical sulci. They are located dominantly in the vicinity of the synapses and are surrounded by astrocytes and microglia. If the form of the disease is more serious, their prevalence and size are also greater. The molecular structure of these pathologies is actually under intensive investigations. By the help of the latest techniques of investigation, these structures, as well as the physical and biochemical nature of their generation, which are fundamental for AD, become more and more transparent.

Equation 1/..... $Fe^{3+} \xrightarrow{A\beta} Fe2^{2+}$ (very aggressive, strong oxidans, toxic)

Equation 2/..... $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^-OH + {}^*OH$

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The dominant material which builds the plaque is in fact the conglomerate of amyloid molecules in form of monomers, dimers, trimers, and fibrils. Among these units there are many advanced glycation end products (AGEs), metal ions (*i.e.* Fe^{2+}, Fe^{3+}, Cu^{2+}, Cu, Zn) and nanoparticles of iron oxides (ferrihydrite, magnetite, ẅustite). The plaque is primarily a conglomerate of amyloid fibrils. Additionally arrived or *in situ* generated, AGEs make strong intertwined intermolecular cross-linking structures, which induce the amyloid beta molecular condensation by bringing them closer one to another, or by folding them with diminution of the spacing between N and C molecular end [4,6,9].

AD is a polygenetic disease. The whole complex of genes induce its early (EOAD: early onset AD), or late more frequent form (LOAD: late onset AD). EOAD or autosomal dominant familial form of AD appears as a rule before the age of 65. The prevalence of this form is under 5% among all cases of AD. Primarily, this form is based on the mutations of these genes: *APP* chr.21q21, *PSEN1* (presenilin 1) and *PSEN2* (presenilin 2). Presenilin is a component of gama secretase, and mutations in this gene are a strong risk factor for AD. *BACE1*, chr.11 (beta secretase) cleaves Aβ segment in the APP (amyloid precursor protein). Gama and beta secretases separate from APP the complete Aβ fragment and induce its significant growth in the local extracellular space. This growth is the crucial factor in the AD pathophisyology. The sporadic form of AD (late onset AD-LOAD) has a prevalence of about 95%. Here, the dominance of *APOEε4* alele is important, which leads to decreased binding of Aβ, to the decrease of its transport to astrocytes and its insufficient degradation [9].

ADAM 10 gene is responsible for the α-secretase activity. Two mutations in this gene weaken the cleaving function of this enzyme, thus, the complete Aβ after this has its full effect. Also, there is no generation of the important protective fragment which protects and regenerates brain cells. The gene is located in the position 15q21.3. It is evident that the Aβ increased concentration in the brain tissues is dependent on its (Aβ) elevated production or on its decreased desintegration and elimination [9-13] .

The importance of the elevated iron concentration in the amyloid plaque has been foreseen for decades. However, only the newest highly sophisticated methods, which enable the exact definition of iron forms and their concentrations, give answers to a series of questions, until now unanswerable. Recent investigations by these methods and tehniques indicate that Aβ has a strong redox potential, and in the plaque range aggregates and binds iron ions, at the same time reducing non-toxic Fe^{3+} (mostly in the form of ferrihydrite) into toxic Fe^{2+} in the form of magnetite and ẅustite. The aim of the presented review is to give the most recent knowledge about this problem area, especially about the possible role of AGEs compounds in the Fe^{3+} reduction [1,6-8].

AGEs compounds and amyloid plaque formation

AGEs are a large heterogenous group of chemical compounds of endogenous and exogenous origin, fundamentally generated by the nonenzymatic binding between the carbonyl groups (C=O) of the reducing sugar (*i.e.* glucose, fructose, ribose) and amino groups (NH_2) of the protein residues side chains. This process is called glycation or nonenzymatic glycosylation, or browning reaction. It is ever more evident how important these compounds are in neurodegenerative diseases, especially in AD. These compounds are believed to lie in the fundamentals of aging. In fact they manifest their harmful effects in two ways. One way consists of their binding to the so called RAGE receptors (RAGE: receptor for AGEs) with the induction of events which lead to harmful damages of many vital structures. The other way is based on their ability to generate cross-links between protein molecules, inducing alterations in their function and irreparable, as a rule, fatal damages. The plaque is primarily composed of proteins. AGEs, with the presented possibility to bind to these molecules, induce characteristic structural changes in the complexity of these connections, and can in all cases influence their function with the appearance of pathological reactions. The question is whether these structural changes have an impact on the ability of Aβ to increase the intensity of Fe^{3+} reduction, or whether AGEs by themselves accelerate the reduction through direct influence. This question requires the answer (***Fig. 1,2***) [14-18].

Iron metabolism and Fe^{3+} accumulation in the neuropil

Ingested by foods, Fe^{3+} passing through the stomach and duodenum, is absorbed on the intestine wall (intestine absorptive cells) where through the action of ferric reductase (duodenal cytochrome b) it is reduced into Fe^{2+}. After the absorption by enterocites, the reduced form is transported by DMT1 (divalent metal transporter 1), and after binding to FPN1 (ferroportin 1), and through the action of hephaestin (membrane-bound ferroxidase), becomes oxidized in Fe^{3+}. FPN1 induces the exit of Fe^{3+} into the blood (duodenum and upper jejunum) where it promptly binds to the transport protein transferin. One transferin molecule binds and transports two Fe^{3+} ions. In the blood brain barrier (BBB) region, the loaded transferin binds to the TfR receptor on the luminal side of the endothelial cells. Through the endocitotic mechanism, the complete TfR-Tf-Fe^{3+} complex enters the cytosol of the endothelial cell. The ferric reductase enzyme, located on the endosome wall, reduces Fe^{3+} into Fe^{2+}. Liberated Tf returns to the blood, and Fe^{2+} bound to DVT1 receptor (divalent metal transporter1), exits from the endosome into the cytosol and after that through ferroportin (FPN1) exits into the neuropil (interstitium). Now, there are two possibilities: Fe^{2+} can directly enter the Fenton reaction generating Fe^{3+}, *OH, and $^{-}$OH; or, after the contact with APP and CP (ceruloplasmin) on the adjacent astrocyte membrane, Fe^{2+} becomes oxidised in Fe^{3+} and enters the amyloid plaque complex. On the other hand, by binding with Tf, Fe^{3+} enters through TfR into the neuron. Not analysing all aspects of these events , the Fe^{3+} route to the plaque is presented, where attracted and subsequently bound to Aβ (metal binding domain) it is reduced into the toxic redox-active Fe^{2+}. As previously stressed, Fe^{2+} promptly enters the Fenton reaction. The iron redox cycle continues, and the accumulation of reactive oxyen species (ROS), especially *OH, becomes a continuing cause of the tissue damage which is the substrate for AD [1,2,4-6] .

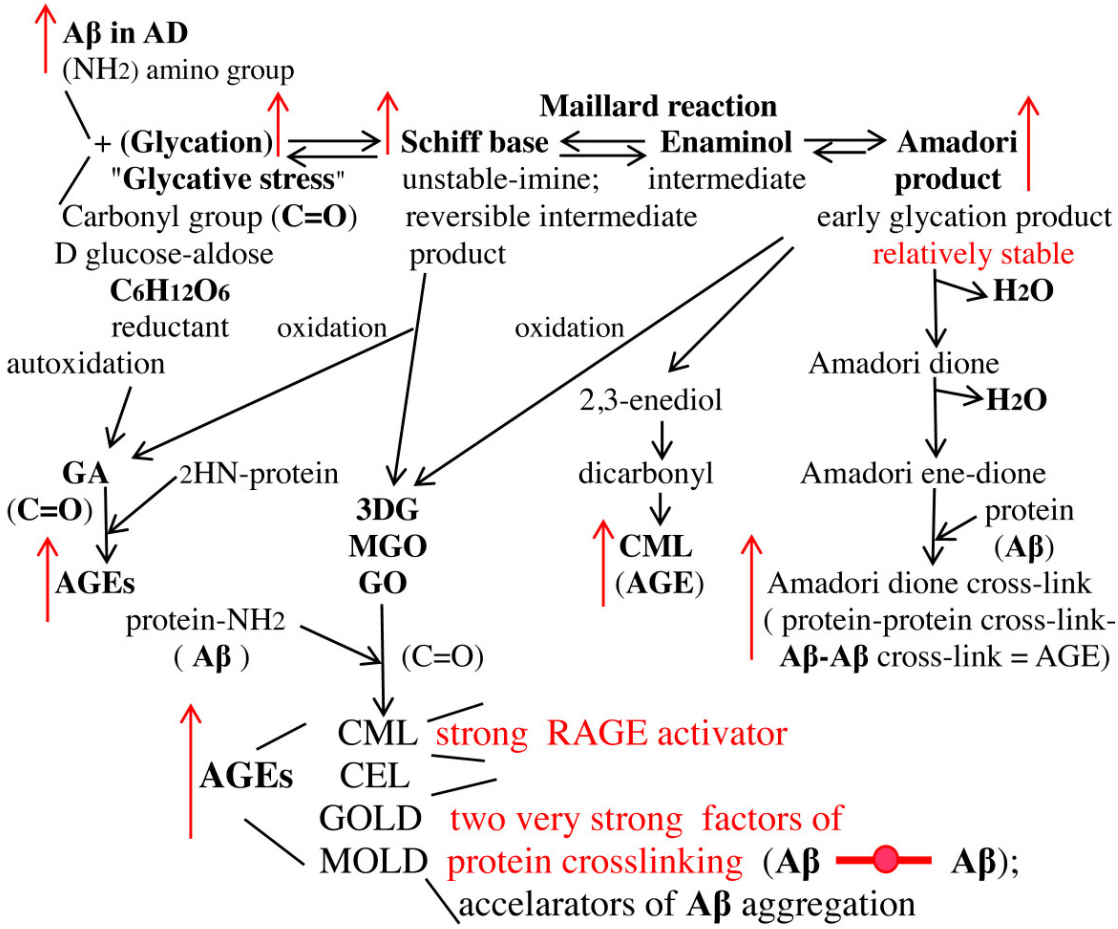

Fig 1. **Schematic presentation of the glycation process and AGE formation**

Aβ, amyloid beta; AD, Alzheimer's disease; glycation, nonenzymatic glycosylation; GA, glyceraldehyde; 3DG, 3-deoxyglucosone; MGO, methylglyoxal, reactive α-ketoaldehyde; GO, glyoxal; CML, N^{ε}-(carboxymethyl)-lysine; CEL, N^{ε}-(carboxyethyl)-lysine; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer; AGE, advanced glycation end product; RAGE, receptor for AGEs. Maillard reaction is a nonenzymatic chemical reaction between amino groups (NH_2) of proteins and carbonyl groups (C=O) of reducing sugars. Glycative stress, overwhelming glycation; exogenous AGEs, dietary or preformed, generated in food; endogenous AGEs,occur in the body, mainly in the bloodstream, During glycative stress there is an AGE overproduction; AGEs are accelerators of Aβ aggregation; The red arrow indicates the increase (↑) or decrease (↓) of the quantity or activity of the appropriate symbol next to the arrow.

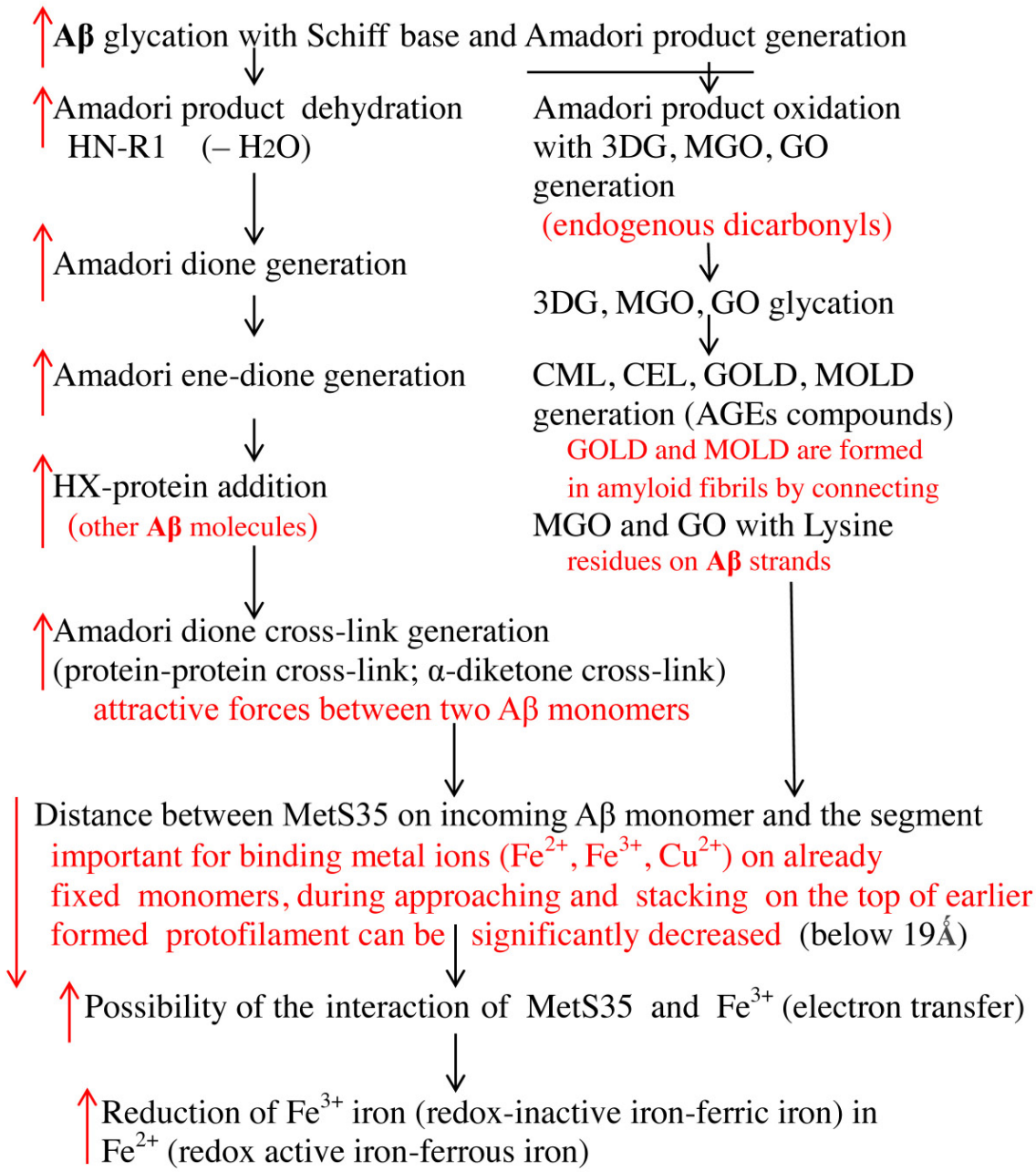

Fig 2. **Schematic presentation of the possible interaction between MetS35 on the β2-strand of the incoming monomer and Fe^{3+},Cu^{2+} on the β1-strand of the tip monomer of already formed part of included protofilament**

Glycation, non-enzymatic glycosylation, covalent bonding between protein or lipid molecule with sugar molecule without assistance of an enzyme; Schiff base, chemical compound that contains a functional group composed of C=N, where N is attached to an aryl or alkyl group, imine; Amadori product, intermediate in glycation and AGEs generation, relatively stable; 3DG, MGO, GO, endogenous dicarbonils; CML, N^{ε}-(carboxymethyl)lysine; CEL, N^{ε}-(carboxyethyl)lysine; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer; MetS35, methionine35; Fe^{3+}, ferric iron; Fe^{2+}, ferrous iron; Cu^{2+}, copper kation; The red arrow indicates the increase (↑) or decrease (↓) of the quantity or activity of the appropriate symbol next to the arrow.

Fenton reaction chemical background

In the course of this reaction, the toxic redox-active ion Fe^{2+} (ferrous iron) by hydrogenii peroxidi (H_2O_2) oxidises (loss of an electron) into the non-toxic redox-inactive Fe^{3+} ion (ferric iron), with the parallel formation of the very aggressive hydroxyl radical (*OH) and hydroxyl ion ($^{-}$OH). This reaction was described for the first time more than 115 years ago by Dr. HJH Fenton, and although at first glance very simple, it is even today the object of discussions and analyses. As to its impact within the AD pathophysiology, it is increasingly becoming a generally recognised component of these compound events, thus the object of intensive investigations. The entrance of Fe^{2+} into the reaction is described in the previous chapter. The problem of the other reaction component, H_2O_2, has also been mostly solved. NADPH oxidase and mitochondrial electron transport chain (ETC) are the source of free electrons, which, reacting with oxygen molecule (O_2), generate aggressive superoxide radical (O_2^{*-}). Binding with hydrogen (2H), this radical generates H_2O_2 and O_2. The reaction is catalysed by superoxide dismutase (SOD). Through the influence of catalase and glutathione peroxidase, H_2O_2 is neutralised. If the H_2O_2 production is increased, or its decomposition decreased, the result is an intensified influx of H_2O_2 into Fenton reaction. This is followed by intensified Fe^{2+} oxidation with possible oxidative stress. The generated Fe^{3+}, attracted by Aβ, enters the close amyloid plaque, where it is subjected to the reduction process. The actual question is about the origin of the increased NADPH oxidase activity. Experiments show that the activation of RAGE receptors, either by free Aβ or Aβ-AGE, has a crucial role in this activity. The generated *OH as a powerful oxidant attacks the neighbouring molecules, especially the lipid membrane components. This is followed by lipid peroxidation with all destructive consequences (***Fig. 3***) [7,8].

The aim of this study is to present a review survey of the most recent knowledge about the role of amyloid beta peptides and AGEs compounds in the reductive transformation of non toxic redox-inactive Fe^{3+} into toxic redox-active Fe^{2+}. Following the above presented initial information, the author's attempt is to show some views and responses to this issue in the next chapter.

Recent studies for MetS35

Smith *et al.* [3] in their excellent review emphasise the structure of Aβ molecule with the analysis of two crucial positions responsible for the the binding of metal ions and their reduction. Aβ is a peptide mainly composed of 42 amino acids. The part closer to the terminal molecular N end is the place where metal ions bind (metal binding domain). MetS35 (methionine 35) side chain residue on the C-terminal peptide end has a powerful reductive capacity. However, reduction (transfer of electrons onto acceptors) happens only in the case when the distance between MetS35 and metal atoms drops below 19 Å (10^{-10n}). According to the authors, this happens either by Aβ folding in the form of capital U, or by agglomeration of two or more units in the fibrile. In the latter case, MetS35 of one monomer comes into a close contact with the region responsible for metal binding of the other monomer. Fe^{3+} previously bound on Aβ, in this way is reduced into redox-active neurotoxic Fe^{2+}. Accordingly, mechanical agglomeration, *i.e.* Aβ fibrils aggregation, induced by the increasing inflow of new Aβ molecules into the plaque, as well as by arrived or *in situ* generated AGEs by glycation, also induces, most likely in an indirect way, an increased Aβ reductive capacity. The importance of MetS35 in AD pathogenesis is also emphasised by a number of other investigators (***Fig. 4, 5, 6***) [4,19-27].

Vitek *et al.*[28] investigate in their analyses the influence of amyloid glycation on the generation of AGEs and so called β-cross structures. They both lead to increased Aβ fibril aggregation, to structural aggregate changes, and to possibly more expressed interreactions with Aβ bound metal ions and MetS35. The appearance of cross-β-structure obviously increases the Aβ reductive capacity. AGEs are a heterogeneous group of covalently bound compounds, generated by additional restructuring, dehydration, oxidation, or by cleaving of early glycation products (Schiff base and Amadori product). Cross-β-structures are present in a great number on long living aggregated proteins, which also include Aβ. The plaques in AD persons contain three-fold more AGEs than the tissue sample of the healthy investigated control subjects. AGEs are actualy protein-protein crosslinked compounds. Their presence in the plaque elevates the resistence of proteins to proteolysis and accelerates aggregation [16,17,29].

Bouma *et al.*[30], using electronic transmission microscopy, prove that albumin glycation leads to its transformation into amyloid fibrils which contain cross-β-structures. The forming of AGEs by itself causes protein aggregation. The authors of the presented study especially emphasise the dehydration process of the intermediar glycation components important for the forming of AGEs, in essence, the cross linking structures, *i.e.* accelerators of the aggregation process in the plaque. The aggregation of amyloid fibrils is essentially accompained by their condensation. During this process, in the plaque occurs an increase of concentration of two essential reactants, *i.e.* amiloyd bound metal ions and MetS35. The rise of reactant concentration elevates the rate of their chemical interreactions, *i.e.* the mentioned reduction. This is confirmed by the 19 Å spacing (mentioned in the study by Smith *et al.*[3]) which evidently decreases with the concentration rise.

In its early phase, the Aβ protein glycation process leads through Schiff base oxidation, as well as Amadori product disintegration through its enol form, to the generation of reactive free α-dicarbonyl compounds, *i.e.* glyoxal (GO) and methylglyoxal (MGO). The linking of these two compounds with protein (Aβ) lysine generates AGEs compounds, *i.e.* glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD). These compounds both belong to the imidazolysine group. Essentially, they evolve by the preliminary reaction of two lysine residues (on two Aβ monomers) and one dicarbonyl molecule (especially MGO). After the formation of the labile Schiff base (labile diimine cross-link), the second dicarbonyl molecule (especially MGO) becomes chemically linked to this complex. Subsequently, through the cyclization reaction, the cross-linking compounds (GOLD and MOLD) are generated. They are both strong cross-linking factors of amyloid protein molecules in the plaque. The final result of this process is a certain alteration of connected structures. These compact compounds undoubtfully elevate the plaque stiffness, and according to investigations, also increase the amyloid reductive capacity (the mutual approaching of MetS35 and metal binding domain). Amadori product dehydration through amadori dione and amadori ene-dione, and by binding with the adjacent protein (Aβ) side chain (lysine), form the amadori dione cross-ink, which strongly links two adjacent protein molecules. The aggregation process is also increased by the Aβ monomer intrinsic tendency to form dimers, trimers and fibrils, which is all additionally supported by ApoEε4 (LOAD) effects (***Fig. 7***) [16,17,27-32].

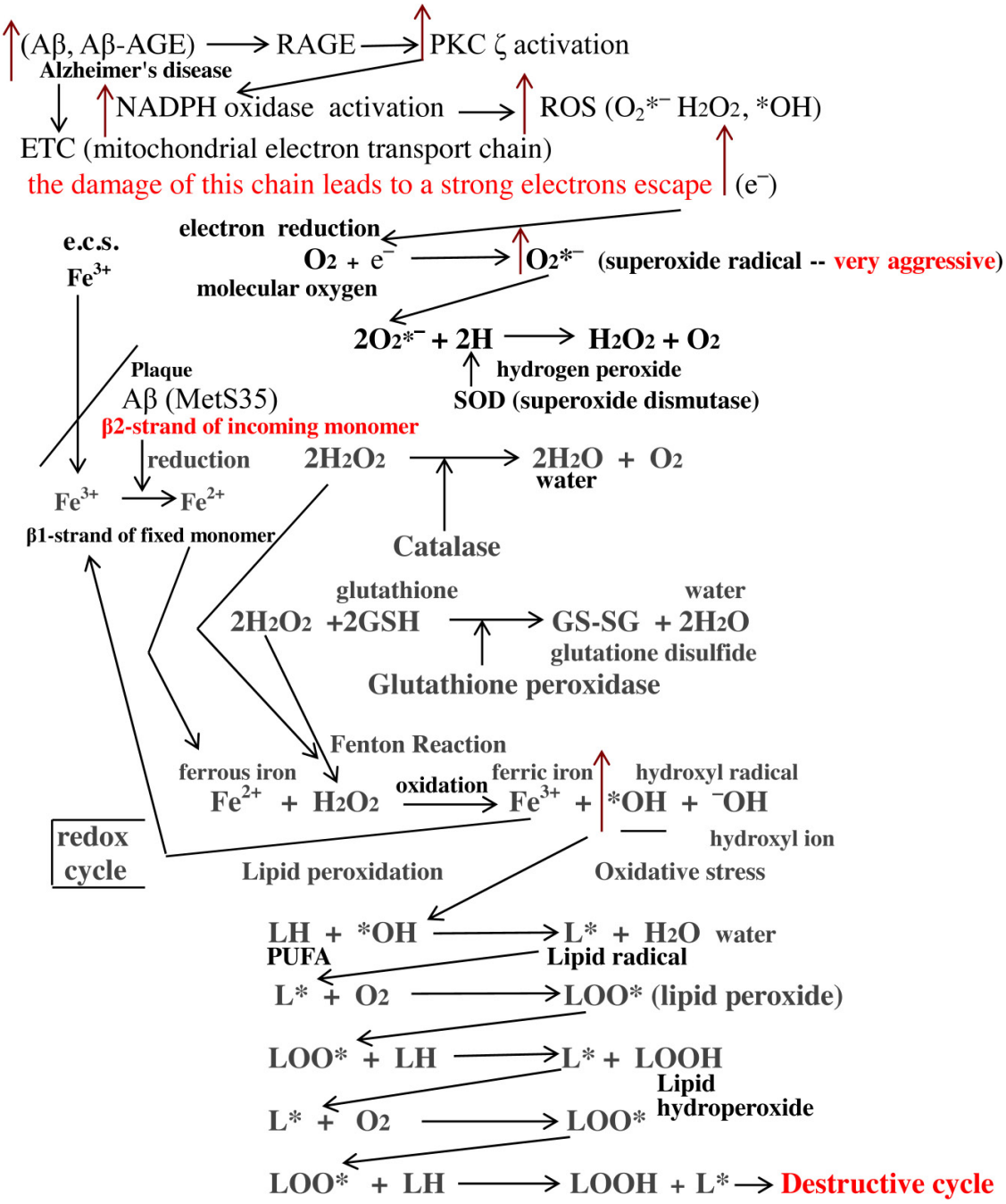

Fig 3. **Schematic presentation of important biochemical processes related to destructive free radical effects on essential molecular structures**

RAGE, receptor for advanced glycation end products; PKC, protein kinase C; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; ROS, reactive oxygen species; O_2*, superoxide radical; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase, strong antioxidant enzyme; catalase, enzyme, protects the cell from oxidative damage; GSH, glutathione peroxidase, enzyme, protection from oxidative damage; PUFA, polyunsaturated fatty acid; lipid peroxidation, oxidative degradation of lipids; Fe^{2+}, Fe^{3+}, iron ions; e.c.s., extracellular space.

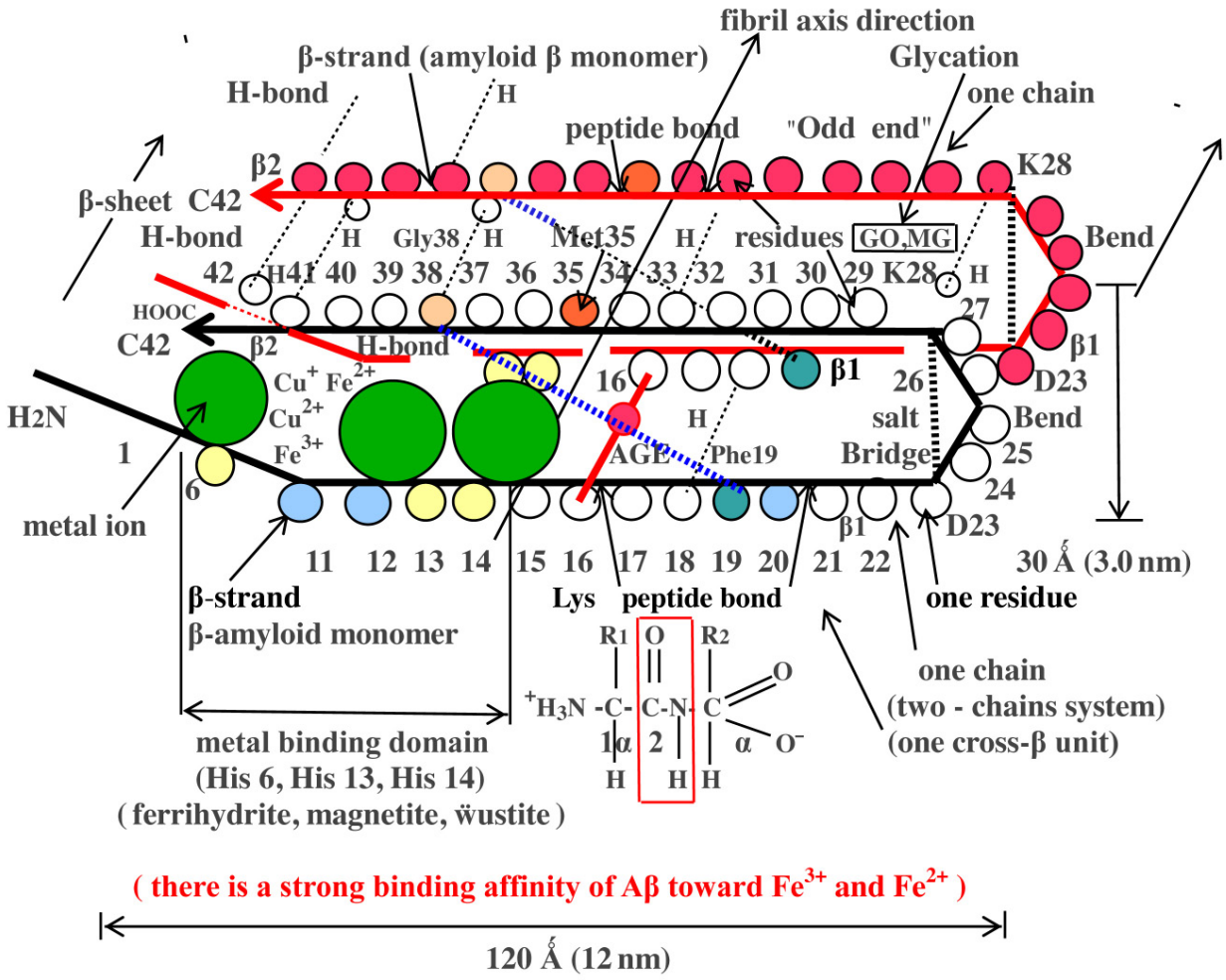

Fig 4. **Schematic presentation of two amyloid beta (Aβ) monomers as a part of one protofilament**

D23-K28, Asp23-Lys28, salt bridge; two stacked Aβ monomers, one presented with blue spheres and other with red; visible H-bonds between adequate positions on adjacent strands; two presented Aβ monomers are segments of a protofilament, all β1 strands form one layer of the β-sheet and all β2 strands form the other layer, in fact β-sheet is a bilayer structure; β-strands lie perpendicularly to the fibril axis; metal bonding domain is presented with green spheres-metal ions; numbered colored spheres represent aminoacids; MOLD is visible with the red connection between two adjacent Lys residues.

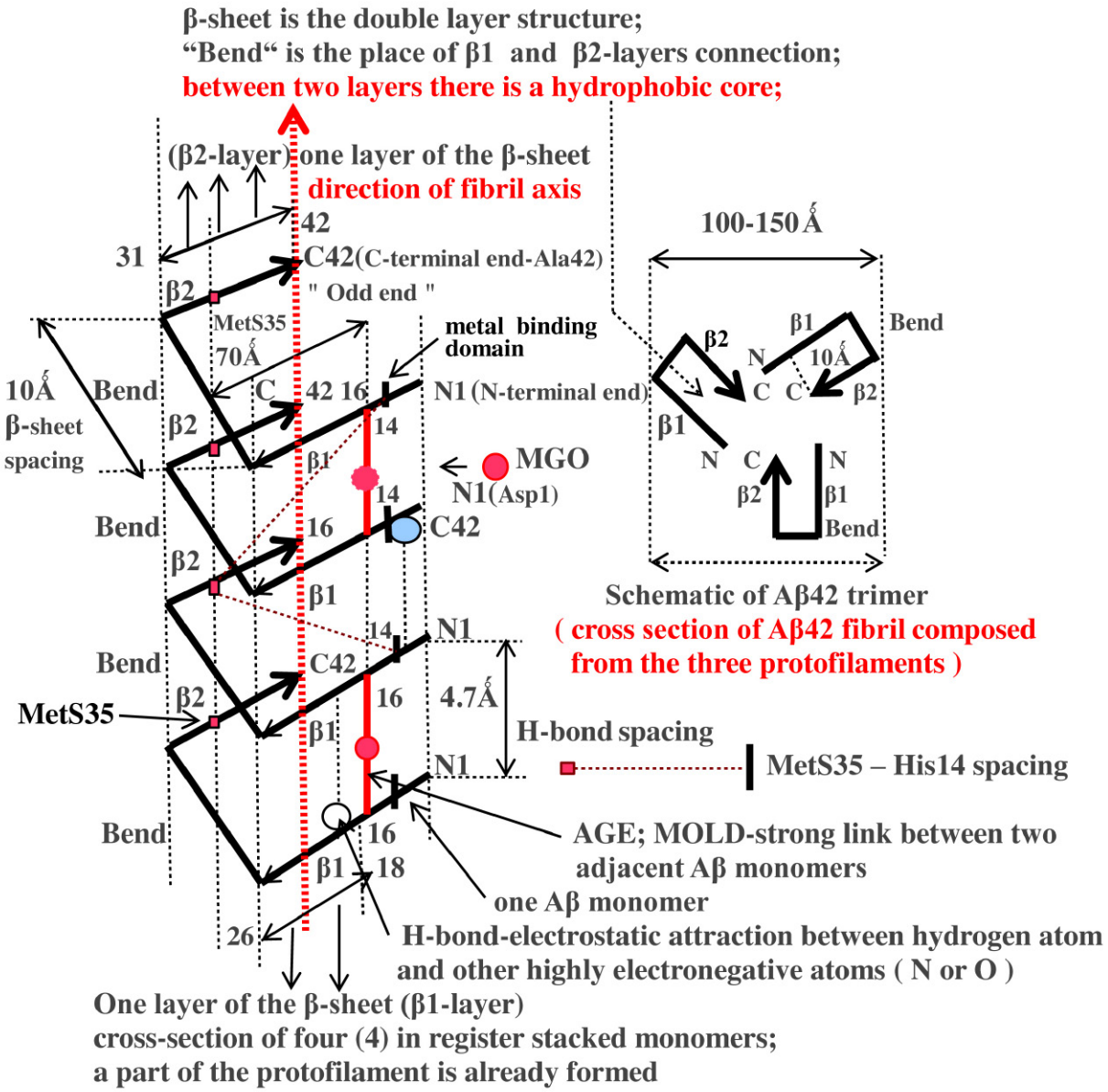

Fig 5. **Schematic presentation of the 3D Aβ(1-42) protofilament essential structure**

β1,β2, monomer's strands; the direction of the fibril axis is indicated by a red arrow; N1, N-terminal end, Asp1; C42, C-terminal end, Ala42; small red square on β2 strand, MetS35; short black line on β1 strand, metal binding domain; MOLD, methylglyoxal lysine dimer; AGE, advanced glycation end product; MGO, methylglyoxal; β-sheet, a flat molecular formation composed of parallel β-strands connected laterally by H-bonds; β1-sheet is composed from residues 18-26 and β2-sheet from residues 31-42.

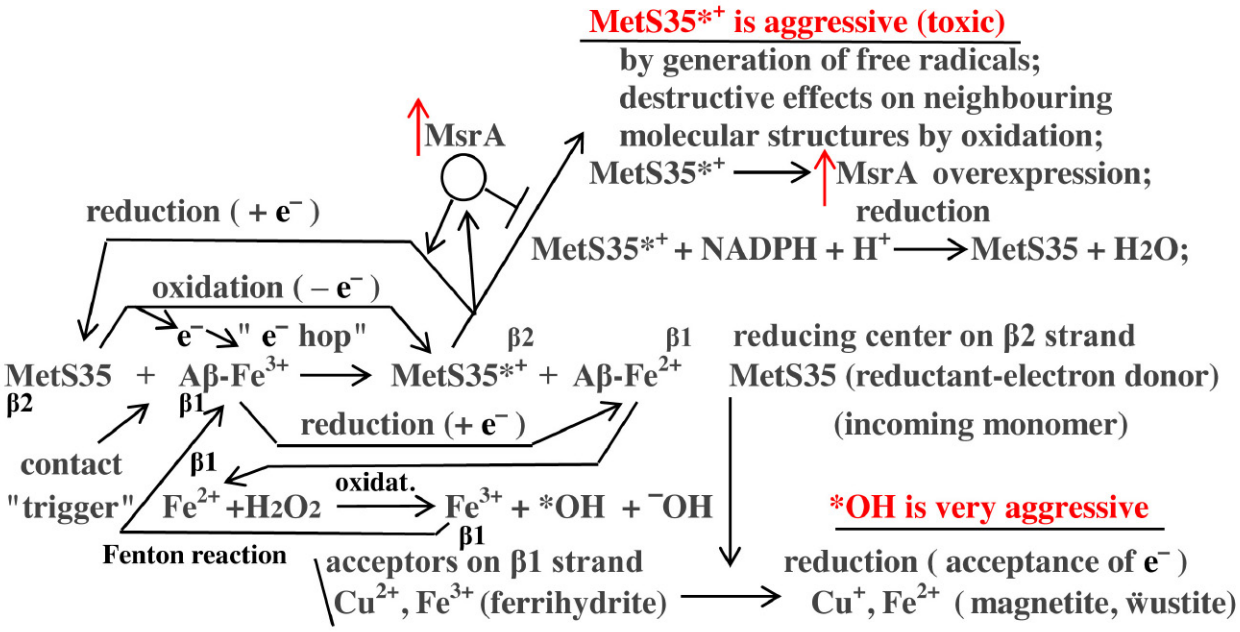

Fig 6. **Two ways of Aβ-mediated generation of destructive compounds**

Metal ions reduction is possible, not before the distance between MetS35 and their location drops below 19 Å. Outer-sphere electron transfer; "electron hop". β1,Aβ-Fe^{3+} and β1, Aβ-Fe^{2+} represent the metal binding domain on β1-strand. Aβ$MetS35^{*+}$ in treated experimental cells induces increase in MsrA mRNA levels and MsrA activity.

MsrA, Methionine sulfoxide reductase type A, enzyme, physiological antioxidant-very useful function; MetS35, methionine 35,reduced form; MetSOx ($MetS^{*+}$), oxidized form, sulfide radical; MetS35 oxidation plays a very important role in AD; *OH, hydroxyl radical; Fe^{2+} , ferrous irin, redox-active iron; ferric iron-redox inactive iron; $^{-}$OH,hydroxyl ion; H_2O_2 , hydrogenii peroxydi; Cu, cuprum,copper; β1 strand, one part of amyno beta monomer where metal binding domain is located; "e^{-} hop", electron crossing (without any mediator) through space from the reducing center to the acceptor; Aβ , amyloid beta peptide; oxidation, electron loss; reduction ,electron gain; H^{+}, hydrogen, proton; NADPH, nicotinamide adenine dinucleotidphosphate, reduced form of $NADP^{+}$; MetS35 sulfur atom has a high tendency towards oxidation. Established interaction between MetS35 (β2-strand of the incoming monomer) and metal binding domain (β1- strand of already fixed monomer) is a trigger for Fe^{3+} reduction. At the same time generated sulfide radical affects, by free radical formation, the neighbouring molecular structures. MsrA (strong reducer) blocks sulfide radical and reduces it in the MetS35. So, one cause of dangerous effects of Aβ is reduced. Ferrihydrite,oxidised form of ferum; magnetite and ẅustite, reduced forms of ferum; Cu^{+}, monovalent cooper; *OH, hydroxyl radical.

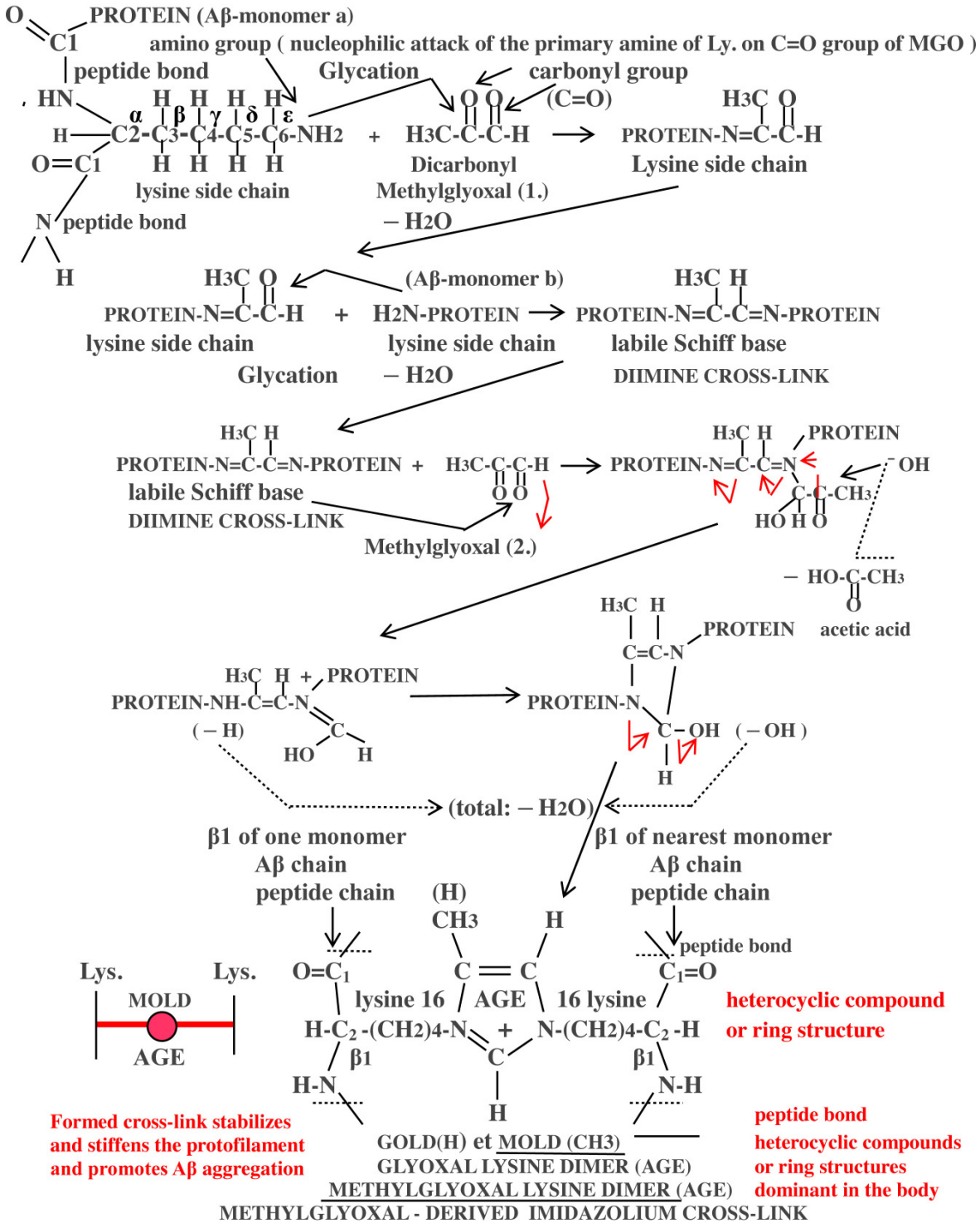

Fig 7. **Scheme of cyclization reaction and GOLD and MOLD formation**

Small red arrow, symbol of electron movement; GO, glyoxal,dicarbonyl compound; MGO, methylglyoxal, dicarbonyl compound; GO and MGO, reactive dicarbonyl compounds, important precursors of protein (Aβ peptide in AD) cross-links; C=O, carbonyl group; glycation, covalent bonding between NH_2 group of proteins with C=O group of reducing sugar or dicarbonyl sugar; AGEs, advanced glycation end products; cyclization, chemical reaction in which chain compounds transform into ring compounds.

Recent studies for Fenton raction

Collingwood *et al.*[6], analysing the material obtained from the plaque core rich in iron, indicate significant values of magnetite biomineral which contains Fe^{2+}. Chemical formula of magnetite is Fe_3O_4, and it is one of the three most frequent iron oxides. It has a strong reductive capacity. By use of high resolution transmission electronic microscopy (HR-TEM), rdg-nergetic-spectroscopy (EDX), and some other sophisticated methods, with the proved, already mentioned high values in the plaque, these authors also indicate the strong neurotoxic effects of magnetite through the participation in Fenton chemistry and through oxidative stress generation. The analysis of obtained results point to the mechanism by which the interaction of Aβ42 and iron can produce elevated concentrations of toxic Fe^{2+} in the plaque. This mechanism also explains the formation of magnetite *in vivo*[5].

Everett *et al.*[7,8] in their recent investigations concerning the cause and consequences of the trovalent iron (ferric iron, Fe^{3+}) accumulation in amyloid aggregates have found very interesting facts, important for understanding the AD background. Using highly sophisticated investigation methods, among them X-ray microspectroscopy, X-ray absorption spectroscopy, electron microscopy, and spectrophotometric iron (II) quantification technology, they have explored the interraction between Aβ and synthetic Fe^{3+} identical to the one stocked in the brain. Experiments indicate the strong capability of Aβ to attract, bind, and accumulate Fe^{3+} in amyloid aggregates, with subsequent reduction in Fe^{2+} redox-active phase. The presence of aluminium (Al) ions (III) elevates the Aβ reductive capacity. Furthermore, experiments have given the explanation of earlier observed high iron values in amyloid plaques, especially of high values of redox-active iron and oxidative stress. Everett *et al.* prove the presence of ferrihydrite (a form of iron oxide) not only in ferritin intracellular stores, but also in amyloid plaques. Ferrihydrite is a form of redox-inactive Fe^{3+}. In the case of damaged ferritin oxidative function, especially in astrocyte degeneration, Aβ attacks the unprotected ferrihydrite and reduces Fe^{3+} into redox-active aggressive Fe^{2+}. On the other hand, when Fe^{3+} enters the plaque in a nonbound form, it partially also transforms into ferrihydrite, but by Aβ it is promptly reduced into Fe^{2+} This indicates that in cases of damaged astrocyte function and high Aβ levels, there is a generation of high Fe^{2+} values with all the earlier mentioned consequences (Fenton reaction ROS, oxidative stress). In the plaque structure, Everett [7,8] detects high values of two valent iron minerals, magnetite and ẅustite, but significantly low values of ferrihydrite, as ferrihydrite has earlier been also transformed into the previous two. The same also applies to damaged astrocytes and altered ferritine function. Everett believes that AGEs do not have a direct influence on the of Fe^{3+} and Cu^{2+} reduction, but AGEs indirectly influence the reduction by elevated aggregation of Aβ and cross-β-structures.

The linking of new Aβ molecules to the existing, already formed cross-β-structures, imperatively depends on the development of intermolecular hydrophobic interreactions, electrostatic interreactions (salt bridges), H-bonding, and concurrent cross-linking through AGEs compounds from the environment or formed from precursors *in situ* (primarily GOLD and MOLD). Through the mutual binding of Aβ-molecular chains which form β-sheet (cross-β-structure), the aggregation process develops, intermolecular spaces decrease, and the possibility rises of the interaction of MetS35 and iron ions fixed on the Aβ chain section, responsible for metal binding (β1-chain). This interaction strongly depends on the interspace of reactants (the critical space is 19 Å). The analysis of the new Aβ-monomer approach indicates that this monomer mainly does not approach perpendicularly to the existing sequence of the forming protofilament (perpendicular approach). In the perpendicular approach, it can be supposed that the reduction rate of Fe^{3+} is minimal. However, in reality this is not the case: actually, because of the dominant oblique approach, the rate is high. The new Aβ-monomer can approach the tip at different angles and with the evident deviation from absolute symmetry (oblique approach). Attractive inter and intra molecular forces attempt to establish a balanced approach. During this adaptation of directions and angles, in the begining there are often small distances between MetS35 (β2-incoming monomer) and Fe^{3+} (β1-tip monomer of already formed protofilament segment) of two approaching monomers. It has to be emphasised that the regular, perpendicular approach of the incoming monomer to the tip protofilament monomer does not ensure MetS35 and Fe^{3+} interaction. In the oblique approach the possible interaction leads to MetS35 oxidation and "electron hop" on Fe^{3+} (reduction). The situation is best explained by reactions in the outer-sphere electron transfer. MetS35 oxidation induced by contact with Aβ1, β1-Fe^{3+} induces MetS35^{*+} (sulphide radical) generation and reduced compound Aβ, β1-Fe^{2+}. The generated Fe^{2+} enters the Fenton reaction with already mentioned consequences. Fe^{3+} generated in the Fenton reaction is again reduced to Fe^{2+} (redox cycle). MetS35^{*+} reduced by methionine sulfoxide reductase type A (MsrA) transforms into the initial reduced form MetS35 (redox cycle). The arrival of the new monomer induces a better interaction of attractive forces between terminal monomers, so the outer sphere electron transfer ("electron hop") is discontinued. The reduction phenomenon can accompany the further protofilament increase (***Fig. 4-10***)[1,3,4,16-19,33-40].

The amyloid fibril is composed on the cross-β-sheet principle, built from β-chains (folded Aβ molecules), positioned perpendicularly against the fibrilar axis. The edges of neighbouring strands are crosslinked by H-bonds. Residues 25-29 form the chain knee, *i.e.* the connection of two molecular paralel layers of one β-sheet (a single cross β-unit). Residues 1-24 belong to one layer and 30-40 to the other. In the hydrophilic space between the two mentioned molecular layers there is an electrostatic link of the residue Asp23 on one side and Lys28 on the other. Hydrophilic interactions through the mentioned space are also present between Phe19 with Val36, and Leu34 with Ile 32. The stated hydrophilic links, as well Asp23-Lys28 bridge, strenghten the existing structure (***Fig. 4,5***)[38,45].

As mentioned above, a number of researchers point to the strong correlation of glycation intensity, especially glycative stress, with AGEs concentration in the plaque and amyloid aggregation dynamics. It is evident that AGEs compounds, either of exogenous or endogenous origin, influence the process of Fe^{3+} reduction and the consequent generation of the dangerous MetS35^{*+} and *OH. Although there is yet no proof of their direct effect on the reduction process, there is probably an indirect effect through the agglomeration acceleration and amyloid fibril generation. The assumption of the oblique approach of the incoming monomer to the still forming protofilament tip, and the possible "electron hop" from one (β2-MetS35) to the other reduction center (β1-metal binding domain), should certainly be considered in detail and experimentally evaluated.

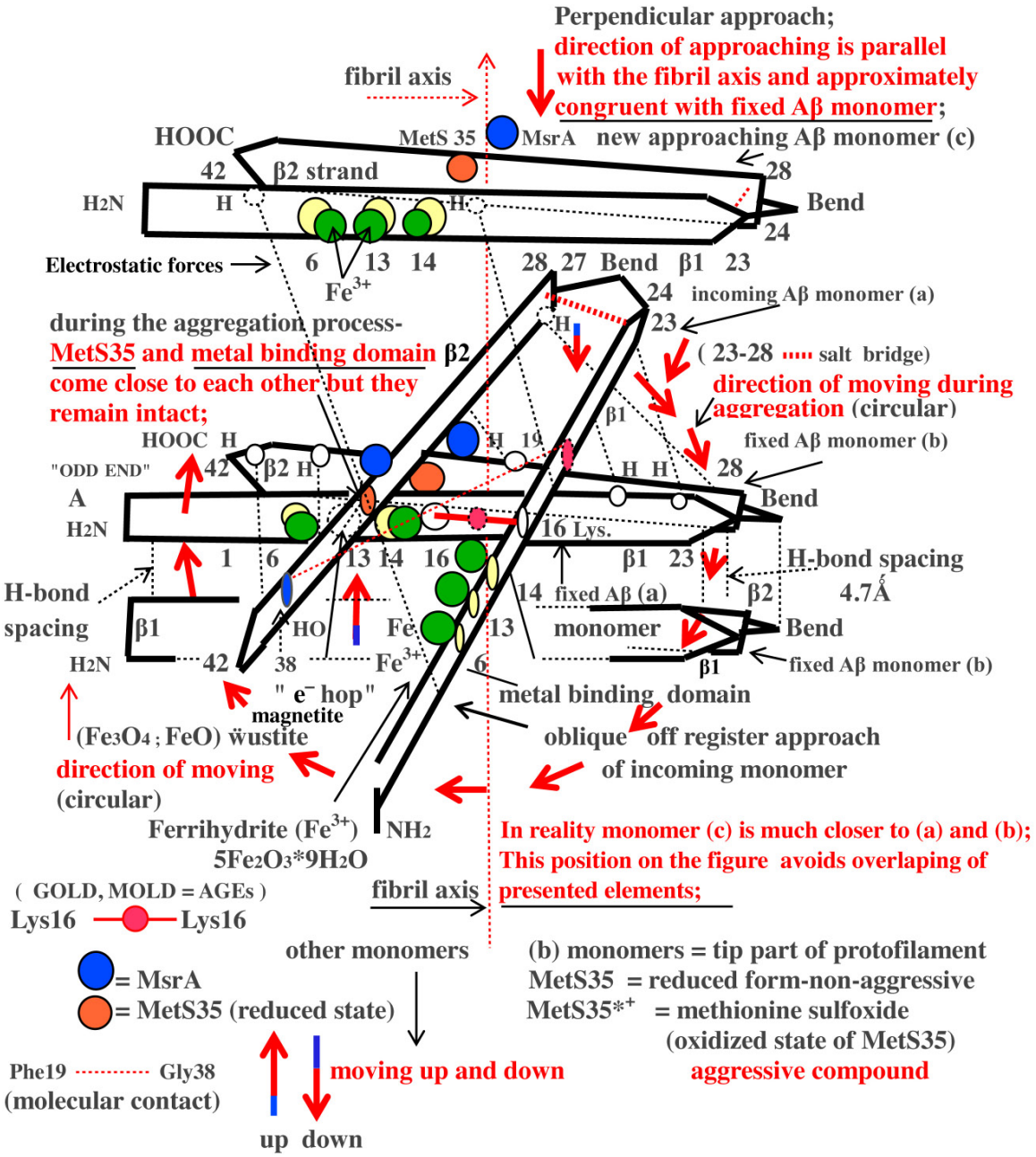

Fig 8. **Schematic presentation of the oblique approach of incoming monomer**

In the oblique approach of the incoming monomer to the odd end of the already formed protofilament, there is a great possibility of close interspace between MetS35 on β2 strand of the incoming monomer and Fe^{3+} on β1 strand of the tip monomer. There is no contact between MetS35 and Fe^{3+} on the already formed part of the protofilament, because the distance between two redox centers is larger. The oblique approach of the incoming monomer to the tip of the already formed protofilament leads to the approach of MetS35 on β2 strand on the incoming monomer to the Fe^{3+} on β1of the already formed protofilament (there is no close contact yet). The restoration of the hydrophobic and H-bond, especially AGEs bonds between β1-β1 and β2-β2 induces a completely close contact between the two mentioned redox centers, "electron hop", and reduction of Fe^{3+} into Fe^{2+}. The arrival of another monomer on the forming protofilament (probably regular and not obliquely directed), interrupts the mentioned contact and reduction. The newly arrived monomer restores the connection with the monomer preliminary bound to the protofilament and corrects its position by inducing the regular arrangement of all units. These events can be repeated uninterruptedly, generating substratums for Fenton reaction and ROS creation; MsrA methionine sulfoxide reductase type A, strong reductant of $MetS35^{*+}$.

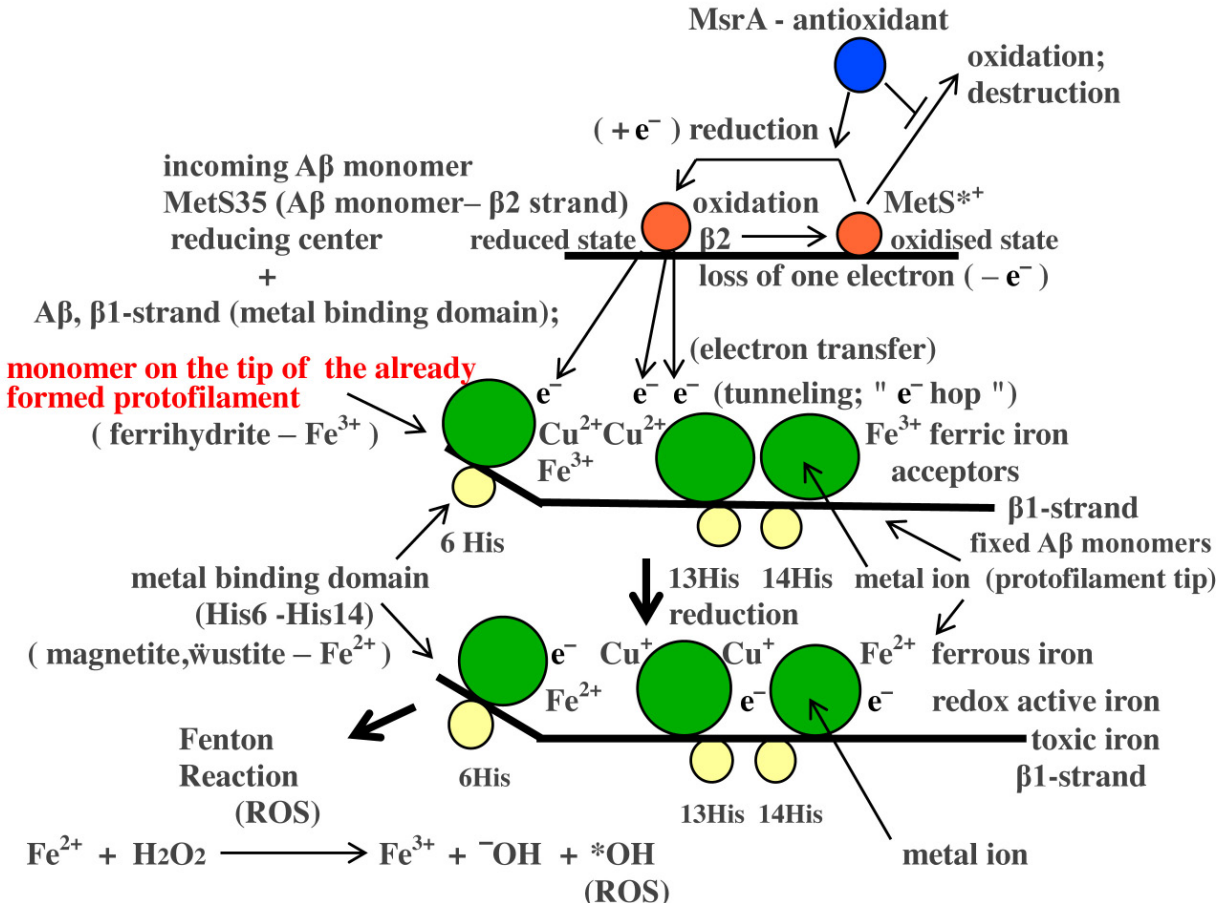

Fig 9. **Schematic presentation of the oxido-reductive process**

MsrA = methionine sulfoxide reductase type A; MetS35, reduced form of methionine; MetS35*+, oxidised state of methionine, methionine sulfoxide, aggressive compound, oxidans; green spheres represent metal binding domain on the β1-strand; His, histidine; ROS, reactive oxygen species; ⁻OH, hydroxyl radical, very aggressive compound; oxidation, adding oxygen or electron loss; reduction, loss of oxygen or gaining of electron; H_2O_2, hydrogenii peroxydi; ferrihydrite, hydrous ferric (Fe^{3+}) oxyhydrite mineral, $5Fe_2O_3*9H_2O$; magnetite, iron II oxide, Fe_3O_4; ẅustite, iron II oxide, FeO.

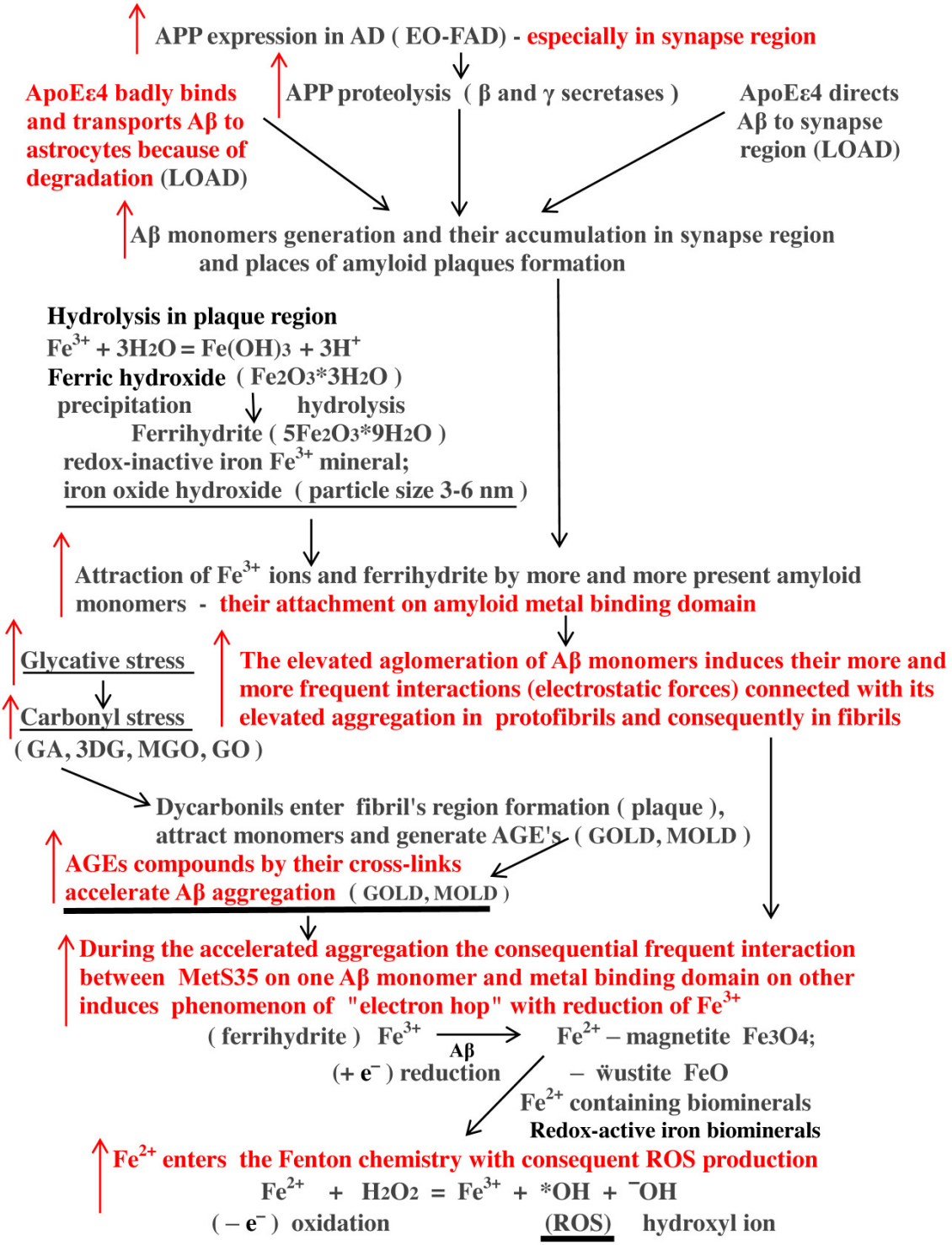

Fig 10. **Schematic presentation of APP proteolysis, Aβ aggregation and Fenton chemistry**

Aβ, amiloid beta; AD, Alzheimer's disease; RCS = reactive carbonyl species (GA, 3DG, MGO, GO); GOLD, glyoxal–lysine dimer; MOLD, methylglyoxal-lysine dimer; AGEs, advanced glycation end products; ROS, reactive oxygen species; EO-FAD, early onset-familial AD; APP, amyloid precursor protein; ApoEε4, apolipoprotein Eε4; γ-secretase, multisubunit protease complex, proteolysis and APP fragmentation; β-secretase, BACE1, proteolysis and APP fragmentation; MetS35, methionine in reduced form; The red arrow indicates the increase (↑) or decrease (↓) of the quantity or activity of the appropriate symbol next to the arrow.

Conclusion

The complex interaction between amyloid beta peptide and AGEs with its accompanying amplified aggregation in amyloid plaques makes up the important component, essential for the phenomenon of oxidative stress and the course of Alzheimer's disease. Amyloid plaque is in fact a composite complex formed primarily from amyloid fibrils mixed with several metals and proteins. Fibrils are composed of several protofibrils and the latter develop by stacking new incoming monomers on the odd end of already formed protofilaments. In the course of this stacking, where the role of electrostatic forces is crucial, the incoming monomer can approach the protofilament tip at different angles and directions. During this approach and the coordination of interrelated stacking, especially in the oblique approach, there is a great possibility of a close meeting of one monomer MetS35 with Fe^{3+} on the other monomer, but without direct contact. The proximity of these two redox centers induces the possibility of "electron hop" from MetS35 onto Fe^{3+} with concomitant reduction and generation of toxic redox-active ferrous iron Fe^{2+}. After entering the Fenton reaction, Fe^{2+} interracts with hydrogen peroxide and the result is the generation of Fe^{3+}, hydrxyl radical (*OH) and hydroxyl ion ($^{-}$OH). *OH induces serious destruction of surrounding molecular structures, especially lipid membranes (lipid peroxidation). Without entering the analysis of other destructive mechanisms induced by amyloid beta in AD, this mechanism may be very important for future investigations of possible therapeutic approaches in the treatment of AD.

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Conflict of Interest Statement

The authors state that performance of this study entailed no issues representing a conflict of interest.

References

1) Huang X, Moir RD, Tanzi RE, et al. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. Ann N Y Acad Sci. 2004; 1012: 153-163.
2) Oshiro S, Morioka MS, Kikuchi M. Dysregulation of iron metabolism in Alzheimer's diease, Parkinson's disease, and amyotrophic lateral sclerosis. Adv Pharmacol Sci. 2011; 2011: 378278. doi: 10.1155/2011/378278. Epub 2011 Oct 12.
3) Smith DG, Cappai R, Bamham KJ. The redox chemistry of the Alzheimer's disease amloid β peptide. Biochim Biophys Acta. 2007; 1768: 1976-1990.
4) Jomova K, Vondrakova D, Lawson M, et al. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem. 2010; 345: 91-104.
5) Khan A, Dobson JP, Exley C. Redox cycling of iron by Abeta42. Free Radic Biol Med. 2006; 40: 557-569.
6) Collingwood JF, Chong RK, Kasama T, et al. Three-dimensional tomographic imaging and characterization of iron compounds within Alzhimer's plaque core material. J Alzheimers Dis. 2008; 14: 235-245.
7) Everett J, Céspedes E, Shelford LR, et al. Evidence of redox-active iron formation following aggregation of ferrihydrite and the Alzheimer's disease peptide β-amyloid. Inorg Chem. 2014; 53: 2803-2809.
8) Everett J, Céspedes E, Shelford LR, et al. Ferrous iron formation following the coaggregation of ferric iron and the Alzheimer's disease peptide β-amyloid (1-42). J R Soc Interface. 2014 Mar 26;11(95):20140165. doi: 10.1098/rsif.2014.0165
9) Barić N. Role of advanced glycation end products in Alzheimer's disease. Glycative Stress Research. 2014; 1: 68-83.
10) Förstl H, Kurz A. Clinical features of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci. 1999; 249: 288-290.
11) Huang Y. Roles of apolipoprotein E4 (ApoE4) in the pathogenesis of Alzheimer's disease: Lessons from ApoE mouse models. Biochem Soc Trans. 2011; 39: 924-932.
12) Koffie RM, Hashimoto T, Tai HC, et al. Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amiloid-β. Brain. 2012; 135: 2155-2168.
13) Suh J, Choi SH, Romano DM, et al. ADAM10 missense mutations potentiate β-amyloid accumulation by impairing prodomain chaperone function. Neuron. 2013: 80: 385-401.
14) Nagai R. Accumulation of advanced glycation end products in AGE-dependent disorders. Medical Hypotheses and Research. 2004; 1: 161-170.
15) Srikanth V, Maczurek A, Phan T, et al. Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. Neurobiol Aging. 2011; 32: 763-777.
16) Iannuzzi C, Maritato R, Irace G, et al. Glycation accelerates fibrillization of the amyloidogenic W7FW14F apomyoglobin. PloS ONE. 2013; 8(12): e80768.doi: 10.1371/journal.pone.0080768.
17) Iannuzzi C, Irace G, Sirangelo I. Differential effects of glycation on protein aggregation and amyloid formation. Frontiers in Molecular Biosciences. 2014; doi: 10.3389/fmolb.2014.00009.
18) Ulrich P, Cerami A. Protein glycation, diabetes, and aging. Recent Prog Horm Res. 2001; 56: 1-21.
19) Hou L, Shao H, Zhang Y, et al. Solution NMR studies of the Aβ(1-40) and Aβ(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J Am Chem Soc. 2004; 126: 1992-2005.
20) Butterfield DA, Sultana R. Methionine-35 of Aβ(1-42): Importance for oxidative stress in Alzheimer disease. J Amino Acids. 2011; 2011: 198430. doi: 10.4061/2011/198430.

21) Butterfield DA, Boyd-Kimball D. The critical role of methionine 35 in Alzheimer's amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity. Biochim Biophys Acta. 2005; 1703: 149-156.
22) Butterfield DA, Kanski J. Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide 1-42. Peptides. 2002; 23: 1299-1309.
23) Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1-42)-induced oxidative stress in Alzheimer disease: Importance in disease pathogenesis and progression. Antioxid Redox Signal. 2013; 19: 823-835.
24) Misiti F, Clementi ME, Giardina B. Oxidation of methionine 35 reduces toxicity of the amyloid β-peptide (1-42) in neuroblastoma cells (IMR-32) via enzyme methionine sulfoxide reductase A expression and function. Neurochem Int. 2010; 56: 597-602.
25) Moskovitz J. Roles of methionine sulfoxide reductases in antioxidant defense, protein regulation and survival. Curr Pharm Des. 2005; 11: 1451-1457.
26) Moskovitz J, Maiti P, Lopes DH, et al. Induction of methionine-sulfoxide reductases protects neurons from amyloid β-protein insults *in vitro* and *in vivo*. Biochemistry. 2011; 50: 10687-10697.
27) Moskovitz J. Methionine sulfoxide reductases: Ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. Biochim Biophys Acta. 2005; 1703: 213-219.
28) Vitek MP, Bhattacharya K, Glendening JM, et al. Advanced glycation end products contribute to amyloidosis in Alzheimer disease. Proc Natl Acad Sci USA. 1994; 91: 4776-4770.
29) Bittencourt Lda S, Zeidán-Chuliá F, Yatsu FK, et al. Guarana (*Paullinia cupana* Mart.) prevents β-amyloid aggregation, generation of advanced glycation-end products (AGEs), and acrolein-induced cytotoxicity on human neuronal-like cells. Phytother Res. 2014; 28: 1615-1624.
30) Bouma B, Kroon-Batenburg LM, Wu YP, et al. Glycation induces formation of amyloid cross-β structure in albumin. J Biol Chem. 2003; 278: 41810-41819.
31) Nagaraj RH, Shipanova IN, Faust FM. Protein cross-linking by the Maillard reaction: Isolation, characterization, and *in vivo* detection of a lysine-lysine cross-link derived from methylglyoxal. J Biol Chem. 1996; 271: 19338-19345.
32) Frye EB, Degenhardt TP, Thorpe SR, et al. Role of the Maillard reaction in aging of tissue proteins: Advanced glycation end product-dependent increase in imidazolium cross-links human lens proteins. J Biol Chem. 1998; 273: 18714-18719.
33) Chang KC, Liang JT, Tsai PS, et al. Prevention of arterial stiffening by pyridoxamine in diabetes is associated with inhibition of the pathogenic glycation on aortic collagen. Br J Pharmacol. 2009; 157: 1419-1426.
34) Page CC, Moser CC, Chen X, et al. Natural engineering principles of electron tunneling in biological oxidation-reduction. Nature. 1999; 402: 47-52.
35) Balland V, Hureau C, Savéant JM. Electrochemical and homogeneous electron transfers to the Alzheimer amyloid-β copper complex follow a preorganization mechanism. Proc Natl Acad Sci USA. 2010; 107: 17113-17118.
36) Kim W, Hecht MH. Generic hydrophobic residues are sufficient to promote aggregation of the Alzheimer's Aβ42 peptide. Proc Natl Acad Sci USA. 2006; 103: 15824-15829.
37) Takeda T, Klimov DK. Replica exchange simulations of the thermodynamics of Aβ fibril growth. Biophys J. 2009; 96: 442-452.
38) Lührs T, Ritter C, Adrian M, et al. 3D structure of Alzheimer's amyloid-β (1-42) fibrils. Proc Natl Acad Sci USA. 2005; 102: 17342-17347.
39) Ahmed M, Davis J, Aucoin D, et al. Structural conversion of neurotoxic amyloid-β(1-42) oligomers to fibrils. Nat Struct Mol Biol. 2010; 17: 561-567.
40) Paravastu AK, Leapman RD, Yau WM, et al. Molecular structural basis for polymorphism in Alzheimer's β-amyloid fibrils. Proc Natl Acad Sci USA. 2008; 105: 18349-18354.
41) Petkova AT, Ishii Y, Balbach JJ, et al. A structural model for Alzheimer's β-amyloid fibrila based on experimental constraints from solid state NMR. Proc Natl Acad Sci USA. 2002; 99: 16742-16747.
42) Petkova AT, Yau WM, Tycko R. Experimental constraints on quaternary structure in Alzheimer's β-amyloid fibrils. Biochemistry. 2006; 45: 498-512.
43) Cheng PN, Liu C, Zhao M, et al. Amyloid β-sheet mimics that antagonize protein aggregation and reduce amyloid toxicity. Nat Chem. 2012; 4: 927-933.
44) Nelson R, Sawaya MR, Balbirnie M, et al. Structure of the cross-beta spine of amyloid-like fibrils. Nature. 2005; 435: 773-778.
45) Wu C, Bowers MT, Shea JE. Molecular structures of quiescently grown and brain-derived polymorphic fibrils of the Alzheimer amyloid Aβ9-40 peptide: A comparison to agitated fibrils. PloS Comput Biol. 2010 Mar 5;6(3): e1000693. doi: 10.1371/journal.pcbi.1000693.