Glycative Stress Research

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Review article

Interaction of methylglyoxal lysine dimer (MOLD) and hydrophobic/hydrophilic forces in the pathophysiology of Alzheimer's disease

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Abstract

***Objective*:** The beta amyloid aggregation process accompanied by the reduction of non-toxic redox inactive ferric iron Fe^{3+} into toxic redox active ferrous iron Fe^{2+}, Fenton reaction acceleration, oxidative and carbonyl stress, makes a crucial link in the pathology and pathophysiology of Alzheimer's disease (AD). In these fascinating events, the composed interaction between advanced glycation end product (AGE) compound methylglyoxal lysine dimer (MOLD) with hydrophobic and hydrophilic residues of the two contacting amyloid (1-42) monomers, generates the complex of hydration and electrostatic forces which complete the aggregation, induce fibrils extension, and generate the phenomenon of reactive oxygen stress crucial for the AD course and harmful AD effects. The aim of the study, which has a review character, is to give an explanation of this composed process, especially of the interplay of MOLD with electrostatic and thermodynamic phenomena, today yet inadequately explained and full of unknown issues data.

***Methods*:** With detailed analysis of published results from a number of recent investigations, reflections and attitudes of eminent world experts who are working on the mentioned problems, the study attempts to give the basis for future investigations in this field.

***Results*:** The results of the study undoubtedly present an important step in understanding the basis of AD; they confirm its polygenetic etiology, the complexity of pathology and pathophysiology which is crucially dependent on the oxidative and carbonyl stress, and give the hope that a solution for its prevention and therapy will be found.

***Discussion*:** In the extensive discussion, which includes the results of a number of investigations, diametrically opposite reflections, and the personal attitude of the author of this study, a comprehensive array of facts has been observed, which need further elaboration.

***Conclusion*:** AD is a serious, chronic, today still incurable neurodegenerative disease. AGEs, especially MOLD, have an important role in its etiology and pathophysiology. There is an increasing accumulation of knowledge on this matter, and the hope about its successful prevention and therapy has a strong foundation.

***KEY WORDS*:** Alzheimer's disease, advanced glycation end products (AGEs), methylglyoxal lysine dimer (MOLD), hydration and electrostatic forces, beta amyloid aggregation, Fe^{3+} reduction, oxidative stress

Introduction

The analysis of the pathology and pathophysiology of Alzheimer's disease (AD) suggests the great importance of amyloid beta (Aβ) aggregation, in other words amyloid fibril extension, accompanied with Fe^{3+} (ferric iron-redox inactive-non-toxic iron) reduction (electron gain) into Fe^{2+} (ferrous iron-redox active-toxic iron), the acceleration of Fenton reaction ($Fe^{2+} + H_2O_2 = Fe^{3+} + {}^*OH + {}^-OH$), and the generation of toxic aggressive hydroxyl radical (*OH), non-toxic hydroxyl ion (OH) and oxidative stress. Fe^{3+} reduction ($\mathbf{Fe^{3+}} \xrightarrow{e^-} \mathbf{Fe^{2+}}$) develops through strong effects of Aβ (residua MetS35 on the β2 chain of incoming Aβ1 monomer is a strong reducer) on this metal fixed on the exactly defined region (His6-His14) on the β1 chain of Aβ2 monomer on the tip of one protofilament of the earlier mentioned fibril. The presented study has the aim to make a detailed elaboration about the key moments connected with today yet insufficiently known interaction between advanced glycation end product (AGE) compound methylglyoxal lysine dimer (MOLD, imidazolium crosslink) with hydrophilic and hydrophobic complex forces during the

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amyloid fibril formation and oxidative stress generation [1-5].

AD is a serious, chronic, neurodegenerative disease connected with the aging process and age. Due to the population aging, especially in highly developed societies (2016, USA, total population – 323,730,000; 2014, USA, 65 years and over – 14.5%; male 19,309,019, female 25,874,360), AD incidence (2014, USA: 148/100,000, 470,686 per year) and prevalence (2014, USA: 1.63%, 5,200,000 per year) are increasing. Costs connected with prevention and treatment have enormously increased (2016, USA: estimated 236 billion US$). AD, in reality, is a polygenetic disease with a rather unclear etiology. For its onset and course, the great importance of advanced glycation end products (AGEs) is more and more evident. AD appears in two forms: EOAD (early onset AD) and LOAD (late onset AD). EOAD, or autosomal dominant familial form, appears before the age of 65. Its prevalence is under 5% of all cases (mutations in *APP* gene-chr.21.q21; *PSEN1* gene-chr.14q24.3; *PSEN2* gene-chr.1q42.13; and *BACE1* gene-chr.11q23.3). LOAD (late onset AD) appears after 65 years of life. Prevalence is 95% of all cases. It is the consequence of two mutations in *ADAM10* gene-chr.15q21.3 and the dominance of *APOEε4* gene (*APOEε4* allele-chr.19q13.2). To sum up, it is typical for AD that the mutations in *APP* gene induce the amyloid precursor protein (APP) elevated production, and subsequently its proteolysis and fragmentation. This occurs by the increased activity of β (BACE1; Beta-secretase1; beta -site amyloid precursor protein cleaving enzyme1) and γ (PS-1, PS-2) secretases. The dominance of the *APOEε4* gene leads to the decline of the Aβ protein transport into astrocytes and its insufficient degradation. Two mutations in the *ADAM10* gene impair the α-secretase activity leading to the decline of cleavage Aβ fragment of APP and the reduced formation of neuroprotective residuum, which repairs brain's neurons. As a consequence, the complete toxic Aβ fragment is released into the cytosol. In reality, AD is the result of increased Aβ production and decreased disintegration [1,2,6,7].

It is important at the beginning to emphasize that this study is not involved in the analysis of the second characteristic of AD, intracellular accumulation of neurofibrillary tangles of τ protein.

Direct attention is given to the earlier mentioned aggregation. In the first phase of the established contact between the new incoming monomer and fixed monomer on the tip of the protofilament (approaching and docking), the new formed AGE compound MOLD, by the decrease of the distance between MetS35 (reduced form of the methionine 35) on the β2 chain of the incoming monomer, and the metal binding domain (MBD, His6-His14, strong affinity for Fe^{3+}) on the β1 chain of the fixed monomer, makes an optimal condition for the earlier mentioned reduction (critical distance ≤ 19 Å for electron "hop"). The adequate connection is a condition for successful aggregation and consequent reduction [2].

It is evident that MOLD can be generated not only on the tip of the protofilament, but also between two stacked monomers included in the structure of the earlier formed protofilament. However, this is not associated with Fe^{3+} reduction and reactive oxygen species (ROS) generation. In this case MOLD only strengthens the fibril structure [8].

What are the AGEs? In reality they are a great group of chemical compounds generated into the body or carried into the body by food, today, considered as crucial factors in the aging process and evolution of a number of chronic degenerative diseases. The structure of these compounds is different; but in reality, they are all generated during the process of non-enzymatic glycosilation or glycation, *i.e.* Maillard reaction. The basis of glycation is the chemical connection between the amino group of protein (NH_2) and carbonyl group of reducing sugar (C=O). They act by crosslinking the important protein molecules, by breaking their function, or by attacking specific AGE receptors (RAGE). Their effect is deteriorative as a rule (***Fig. 1, 2***) [1,2,8]. Later we shall present in detail the analysis of Page *et al.* [9] about the mentioned reduction and critical distance 19 Å for the electron "hop" [2].

The contact between two redox centers (MetS35, β2Aβ1 and Fe^{3+}, MBD, β1Aβ2) is reinforced by simultaneous hydrophobic interaction of central hydrophobic residues Leu17-Ala21 (β1/β1) of the approaching monomers (Aβ1/Aβ2). According to its position (Lys16-Lys16), and the great strength of covalent bonds (C-N, 308 kJ/mol; C-C, 348 kJ/mol), MOLD becomes a strong fictitious rotation center (clockwise rotation) of the incoming monomer reinforced by mutual hydrophobic attractive contacts of both Leu17-Ala21 groups, firstly on β1 chains, and then by stronger attractive contacts on β2 chains (Ala30-Leu34), and by new H-bonds and salt bridges (***Fig. 1, 2***) [10-14].

The establishment of a parallel and in-register position of the last monomer, which to a large degree has contributed to the appearance of the new incoming monomer (especially if it is approaching closely in a parallel and in-register direction in relation to the formed protofilament) [12,14], leads to the breaking of reduction (the distance between MetS35 and MBD becomes larger) and to a stronger fixation of the incoming monomer on the fibril structure (locking). The process continues further without breaking. Besides the oblique off-register connection of the incoming and fixed monomer, with a strong MOLD link, there is a hydrophobic attraction which develops between the residues Leu17-Ala21 (central hydrophobic cluster) on both β1 (Aβ1, Aβ2) strands. According to Berhanu *et al* [13], the good shape complementarities of the included hydrophobic surfaces, hydrophobicity, and van der Waals interactions are of great importance. The attraction on β2 strands (Ala30-Leu34) at the beginning, due to the great interspace, is weak, but with the progression of the rotation, it becomes stronger, and the tendency to the parallel and in-register position is increasingly stronger (***Fig. 1, 2***) [2,11,12].

The domination of oblique, non-parallel, off-register approach is suggested by the frequent oxidation of MetS35 with the reduction of Fe^{3+} into Fe^{2+}. In the case of a rare coordinated, parallel, in-register approach, there is no reduction, or the reduction is minimal (distance between MetS35 and MBD, Fe^{3+} > 19 Å). The appearance of MOLD connection with the oblique, off-register approach, and hydrophobic contacts in the Leu17-Ala21 and Ala30-Leu34, have a crucial role in the mentioned rotation and ROS generation. It is clear that new H-bonds and salt bridges help the rotation process (***Fig. 1, 2***) [2,9].

Many scientists emphasize the great importance of endogenously generated MOLD in the AD pathophysiology and pathology. MOLD is a complex compound generated through the glycation and connection of two methylglyoxal (MG, belongs to reactive dicarbonyl compound [RDC]) molecules with two Aβ molecules. The final form visible in a strong covalent bond between the two Aβ molecules makes the basis for the mentioned reduction, the rotation of the incoming monomer and consequent fibril extension [2]. The aim of this study is to give a review of the detailed

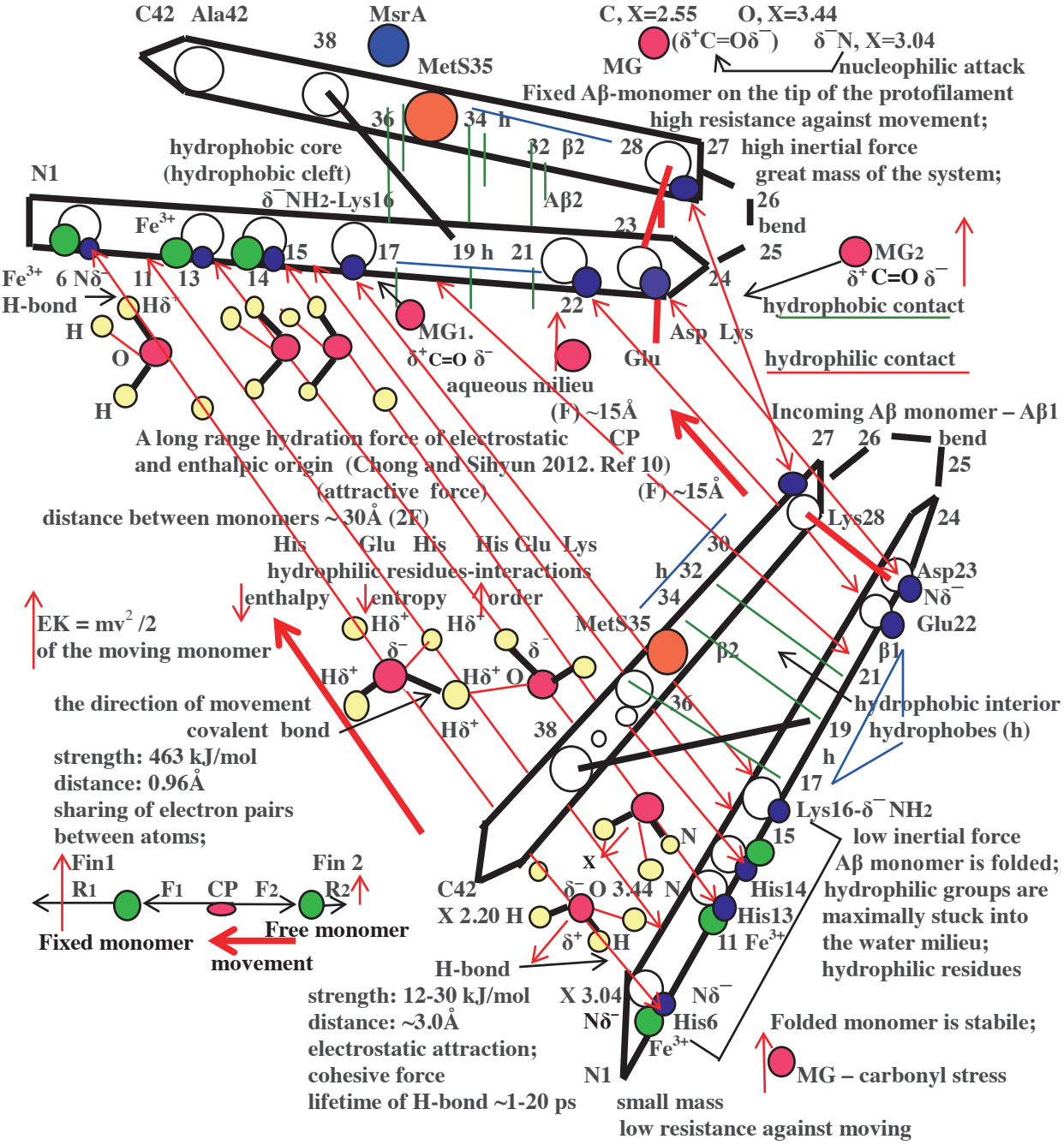

Fig. 1. **Simplified schematic presentation of two Aβ monomers and their mutual approach.**

Aβ, amyloid beta; X, electronegativity; ps, picosecond, 10^{-12} sec; MG, methylglyoxal; EK, kinetic energy of the moving monomer; m, mass; v, velocity; Å, unit of length equal to 10^{-10} m; (F) ~ 15 Å, border of hydrophilic residues influence; CP, contact point of two energetic fields (2F); R1/R2, resistances; F1/F2, electrostatic forces; glycation, reaction between protein NH_2 group and MG C=O group; electrostatic attraction between MG carbonyl group (C=O) and NH2 lysine (Lys16) group – nucleophilic attack; hydrophilic residues belong to hydrophilic exterior; hydrophobic interior-hydrophobic cleft; H-bond – permanent rapid generation and breaking; A, Ala, alanine; L, Leu, leucine; in relation to the lateral attraction, incoming monomer has a dominant tendency to dock on the edge of the protofilament; Leu17-Ala21 and Ala30-Leu34; h, hydrophobic complexes; water molecules are eliminated from the hydrophobic cleft.

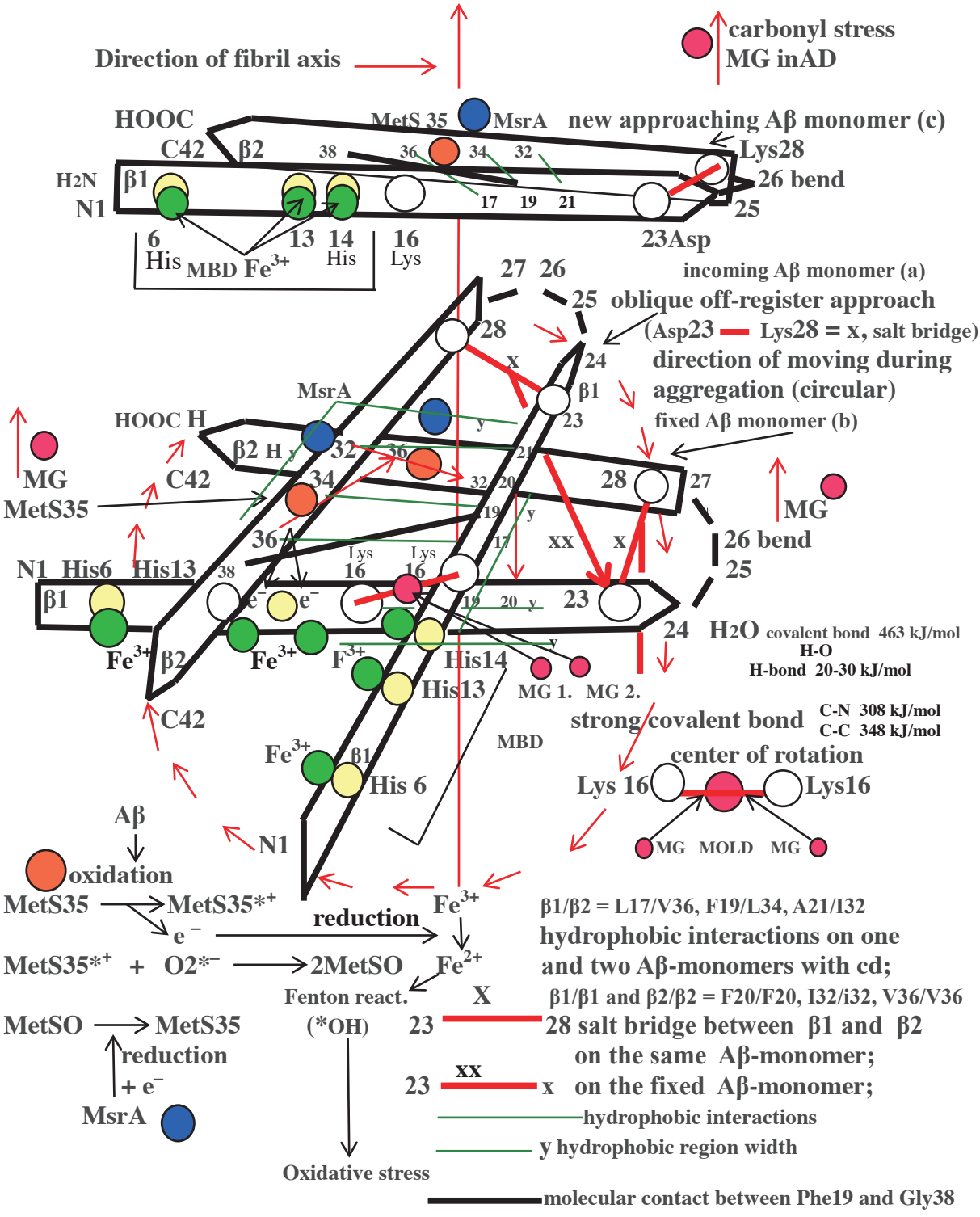

Fig. 2. **Complete contact between two approaching monomers.**

AD, Alzheimer's disease; Aβ, amyloid beta; MG, methylglyoxal; MOLD, methylglyoxal-lysine dimer; MetS35, methionine S35, reduced form of methionine; MetS35*+, methionine sulfide radical-oxidized form of MetS35; MetSO, methionine sulfoxide; MsrA, methionine sulfoxide reductase type A; Fe^{3+}, ferric iron, redox-inactive iron; F^{2+}, ferrous iron, redox-active iron; reduction, gain of electrons or loss of oxygen; during the aggregation process, MetS35 and MBD come close to each other, but they remain intact; MBD, metal binding domain, Lys6-Lys14; incoming monomer deposits to the protofilament, *i.e.* fibril, usually as a monomer; interaction between incoming monomer and the tip of protofilament is more frequent than between isolated monomers; cd, contact distance: β1/β2 ≈ 10 Å; β1/β1 and β2/β2 ≈ 5 Å.

analysis of composed molecular processes in the amyloid plaque, which makes the fundament of AD [2].

Aβ monomer develops through the transmembrane protein APP proteolysis (activity of β and γ secretase enzymes) and the release from the neuronal cell membrane in the local extra-cellular perisynaptic space. Prompt contact with water induces the hydrophobic effect. Around the parts of Aβ molecules, which are situated on the β1 and β2 strands (hydrophobic complexes – Leu17-Ala23 and Ala30-Leu34), originates the water "cage" (solvation shell), which leads to the reorientation of water dipoles and polarization field with strong electrostatic potential.

This is the optimal condition for the generation of long-range attractive forces between monomers and their attraction [1,2].

Without entering the complex analysis of the Aβ effects on the depression of the excitatory transmission on the synapse level, the aberrant characteristics of neuronal activity, and epileptiform discharges on the neuronal network, this study is primarily involved in the AGEs and hydrophilic/hydrophobic forces interactions during the ROS (primarily *OH) generation and oxidative and carbonyl stress [15,16].

Discussion

The analysis of the mentioned events requires knowledge about the ways of MG generation. A well-known way includes glycolysis, pyruvate (ionized form of pyruvic acid, $CH3COCOO^-$) and pyruvic acid (CH3COCOOH), pyruvic acid reduction and MG (CH3COCOH) generation. It is clear that reinforced glycolysis, which is especially accelerated in hyperglycemia, leads to a higher production of this compound (MG). A great number of recent investigations emphasize that MG is the key factor in endogenous AGEs generation, primarily MOLD (***Fig. 3***) [2,17]. During the glycolysis, two intermediate compounds, glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), have a significant importance for MG generation. There is a consensus that this is a crucial way for MG production in the body. By effects of the triose phosphate isomerase (TPI or TIM) enzyme, the DHAP and GAP interconversion occurs, as well as the generation of the labile endiolate phosphate, the elimination of phosphate part ($HPO4^{2-}$), and the occurrence of MG through enolaldehyde (***Fig. 4***). The mentioned authors quote several secondary endogenous ways which generate MG. AD is marked with the rise in Aβ production and its intensive glycation during the early phase of Maillard reaction. The intermediate product of this reaction, the Amadori product, with the influence of a great quantity of oxidants (oxidative stress) typical for AD, oxidizes strongly in MG. Schiff base also transforms into MG – Namiki pathway (***Fig. 5***) [1,2,18,19].

Considering the elevated generation of MG and Aβ in AD, it becomes evident that their elevated concentration in the interstitium can lead to their frequent contact and rise in MOLD generation and intensive Aβ aggregation. This is in congruence with the chemical law that the velocity and intensity of chemical reaction is strongly related to the involved substrate quantity. The mutual attraction between MG molecule ($C\delta^+ = O\delta^-$, carbonyl group) and Aβ ($NH2\delta^-$, amino group) occurs in the aqueous environment, possibly through the influence of electrostatic forces (Coulomb's law). There is a tendency for the addition of the electron rich chemical species to the electron deficient (partial positive charge, δ^+) carbon on the MG carbonyl group (***Fig. 6***) [1,2,20].

The U-shape of Aβ monomer structure is especially influenced by the interaction of hydrophobic residues on the β1 and β2 chain of the respective monomer (folding). Hydrophobic links Leu17/Val36, Phe19/Leu34 and Ala21/Ile32 build this complex stable connection (face-to-face hydrophobic contacts between residues). These connections are especially important for the stabilization of the typical β-sheet structures, components of Aβ protofilament. In these contacts, special importance lies in the exact congruity of hydrophobic surfaces on the same monomer (β1/β2) or on the neighboring monomers (β1/β1, β2/β2). The congruent surfaces are mutually attracted and connected by effects of hydrophobic (<40 kJ/mol; distance varies) and van der Waals (0.4-4.0 kJ/mol; distance 0.3-0.6 nm) forces. The strength of hydrophobic surfaces (located on the internal monomer's part) interaction, connected with the exclusion of water molecules and the formation of hydrophobic cleft, lies in the wide, ramified, non-polar side chains of valine, leucine, isoleucine and phenylalanine. These side chains cannot form H-bonds with water and other polar compounds. The stabilization is also contributed by the electrostatic joints (salt bridge) between residues Asp23 and Lys28, including the new-formed H-bonds. The Aβ monomer U-shape is very important during the contact of the incoming monomer with the fixed monomer. This shape enables the optimal MOLD formation and MetS35 (β2Aβ1) and MBD (β1Aβ2) contact with the accompanied Fe^{3+} reduction and oxidative stress occurrence. It is evident that MOLD, with its strength, contributes to the stability of the U-section of the protofilament (***Fig. 1, 2***) [2,3,11-13].

It is pointed out that during the oblique, off-register approach of the incoming monomer (Aβ1) to the tip of the protofilament, there is a possible formation of a strong covalent bond between Lys16 and Lys 16 of the incoming and already fixed monomers with the formation of AGE compound MOLD (strong covalent bond: C-N = 308 kJ/mol, C-C = 348 kJ/mol, C = N = 615 kJ/mol). Lysine has a polar side chain placed on the surface of the monomer. It is soluble in water. At the same time, two redox centers begin to approach, MetS35 on β2 of the incoming monomer and MBD on the β1 of the already fixed monomer. By reducing the critical distance (19 Å), electrons can jump (electron "hop") and induce the reduction of Fe^{3+} into Fe^{2+} [2,9]. The formation of MOLD (dominant AGE in the body), which is a relatively quick yet step-by-step lasting synthetic complex process composed from several glycations and cyclization, leads to a stronger contact between two hydrophobic centers Leu17 and Ala21 on β1/β1 and the occurrence of the mild rotation (clockwise rotation) of the incoming monomer in the direction of in-register docking. In reality this is the consequence of the tendency of two similar residues on β1/β1 to enter into relation from the initial minimal into the optimal (balanced) contact. It is evident that the process of MOLD formation after the first glycation (MG1 + Aβ1) is accompanied by the attraction of Aβ2 (on ***Fig. 1, 2***. incoming monomer, Aβ1, movable monomer), the second glycation (Aβ1 = MG1 + Aβ2), the formation of the labile Schiff base (Aβ1 = MG1 = Aβ2), and the third glycation (Aβ1 = MG1 = Aβ2 + MG2). This process evidently contributes to the mutual monomers attraction (Aβ1 and Aβ2), and is congruent with the attraction through the newly formed H-bonds. The congruent attraction accelerates the Aβ aggregation. In the moment when H-bonds between the water molecules and the N-atom of hydrophilic lysine disrupts, the possibilities of nucleophilic attack and the glycation between NH_2 protein

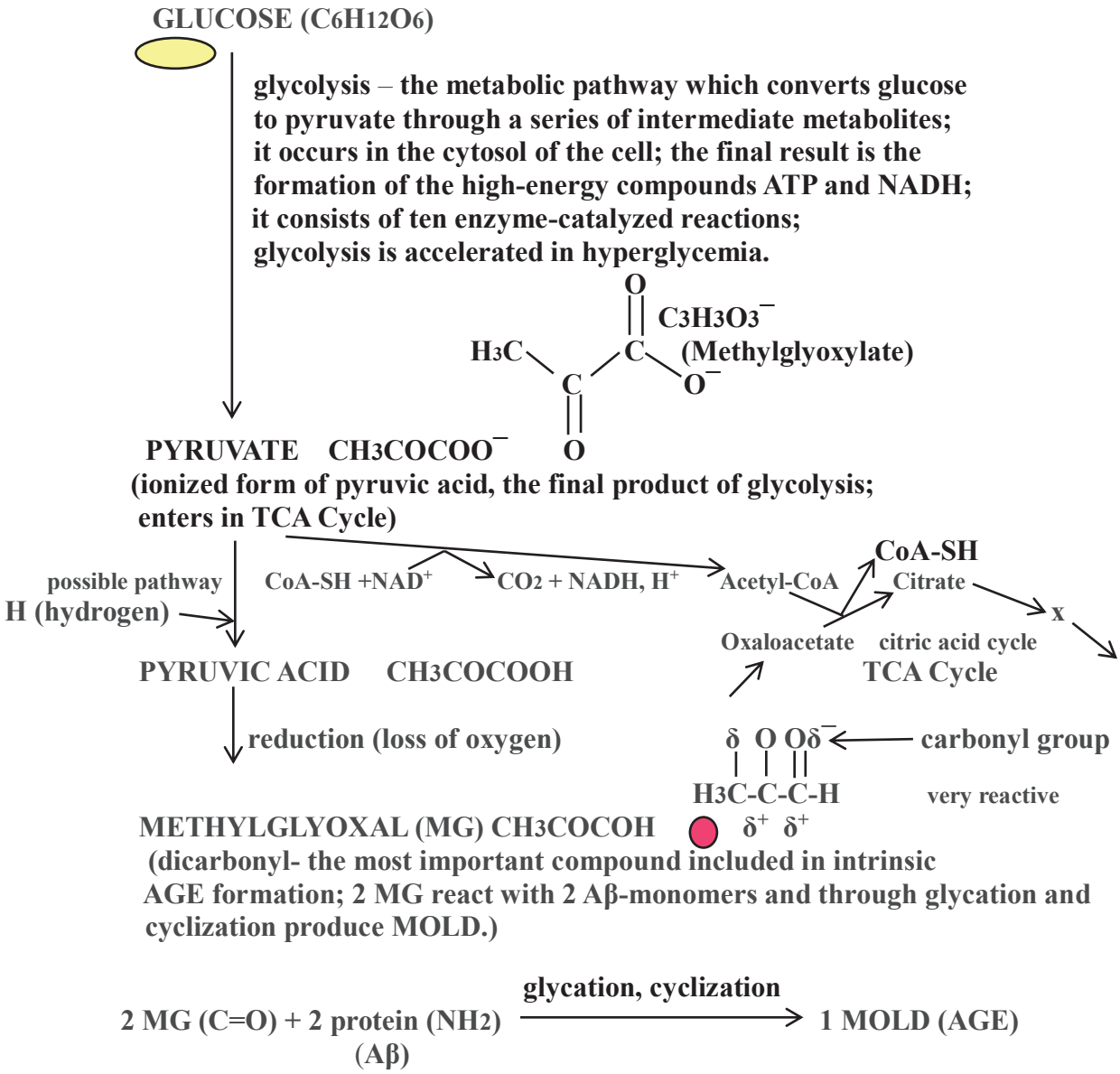

Fig. 3. **Methylglyoxal generation from pyruvic acid.**

Aβ, amyloid beta; MG, methylglyoxal; MOLD, methylglyoxal-lysine dimer; AGE, advanced glycation end product; TCA cycle, tricarboxylic acid cycle, citric acid cycle, Krebs Cycle, series of enzyme catalyzed chemical reactions a key part of aerobic respiration in cells; oxaloacetate and citrate, components of the Krebs Cycle; Acetyl-CoA, one of the most important molecules for metabolism; CoA-SH, coenzyme A; ATP, adenosine triphosphate; NADH, reduced nicotinamide adenine dinucleotide; NAD^+, nicotinamide adenine dinucleotide; δ^+, partially electropositive; δ^-, partially electronegative; C=O, carbonyl group; Krebs cycle produces 2 ATP, 8 NADH, and 2 $FADH_2$ per one molecule of glucose; x, isocitrate.

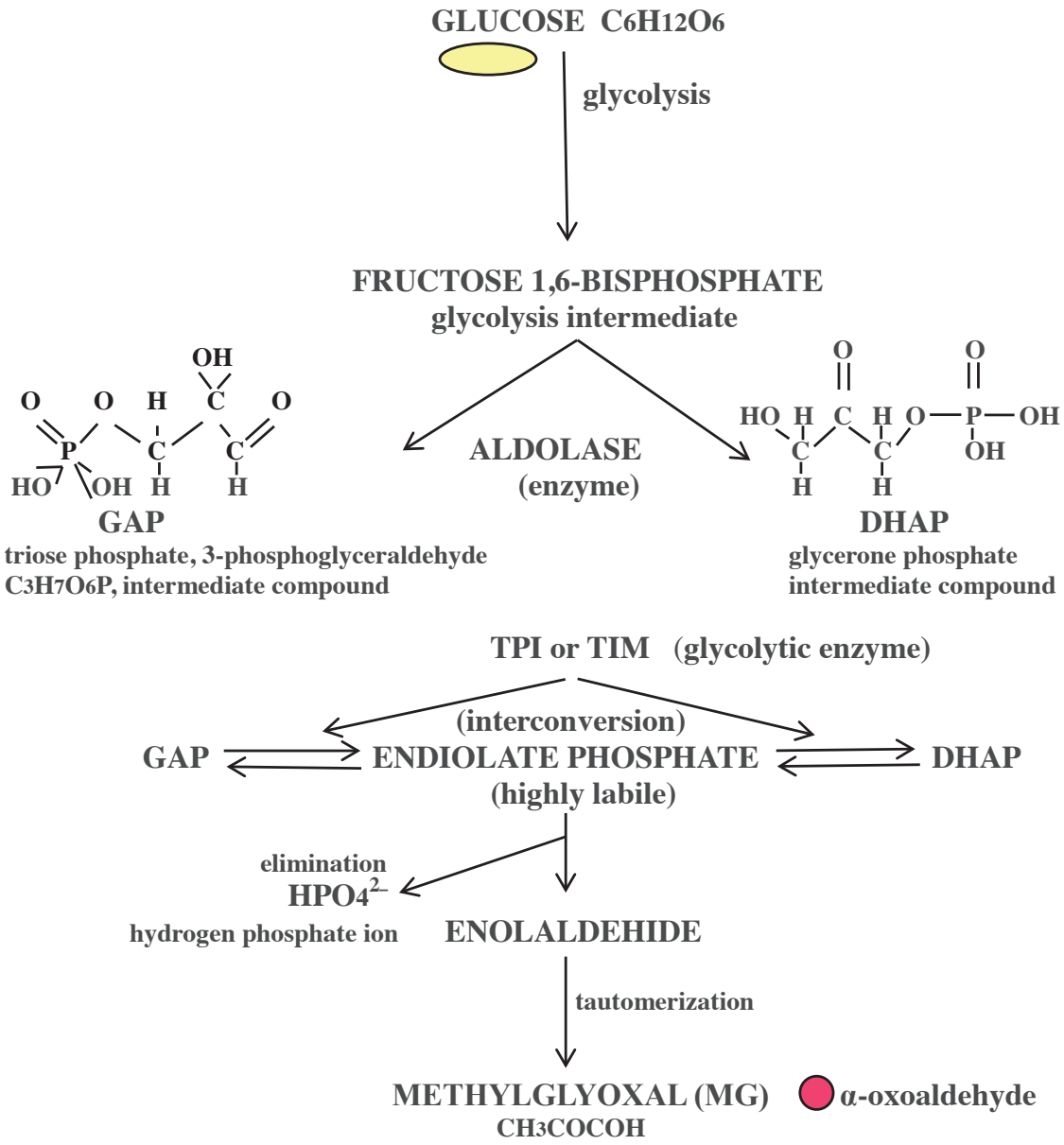

Fig. 4. **Synthesis of methylglyoxal in an elimination reaction by triose phosphate isomerase.**

Methylglyoxal (MG) is a highly reactive and toxic compound; MG has two functional groups: very reactive aldehyde group and electron acceptor ketone group; MG is an electrophilic molecule; MG can initiate glycation reaction; it is the most important glycation agent *in vivo*; it has great affinity for lysine side chain; *in vivo* there is a strong nucleophilic attack by the N atom of NH_2 group of lysine residue to the electrophilic carbonyl group of MG ketone part; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; TPI or TIM, triose phosphate isomerase; HPO_4^{2-}, hydrogen phosphate ion; tautomerization, the chemical reaction interconverting two isomers; H_2O_2, hydrogenii peroxidi; R, protein; there is the consensus that this way is a crucial way for MG production in the body. MG is the key factor in endogenous AGE generation, primarily MOLD; tautomerization, event pervasive in organic chemistry; MOLD, methylglyoxal lysine dimer.

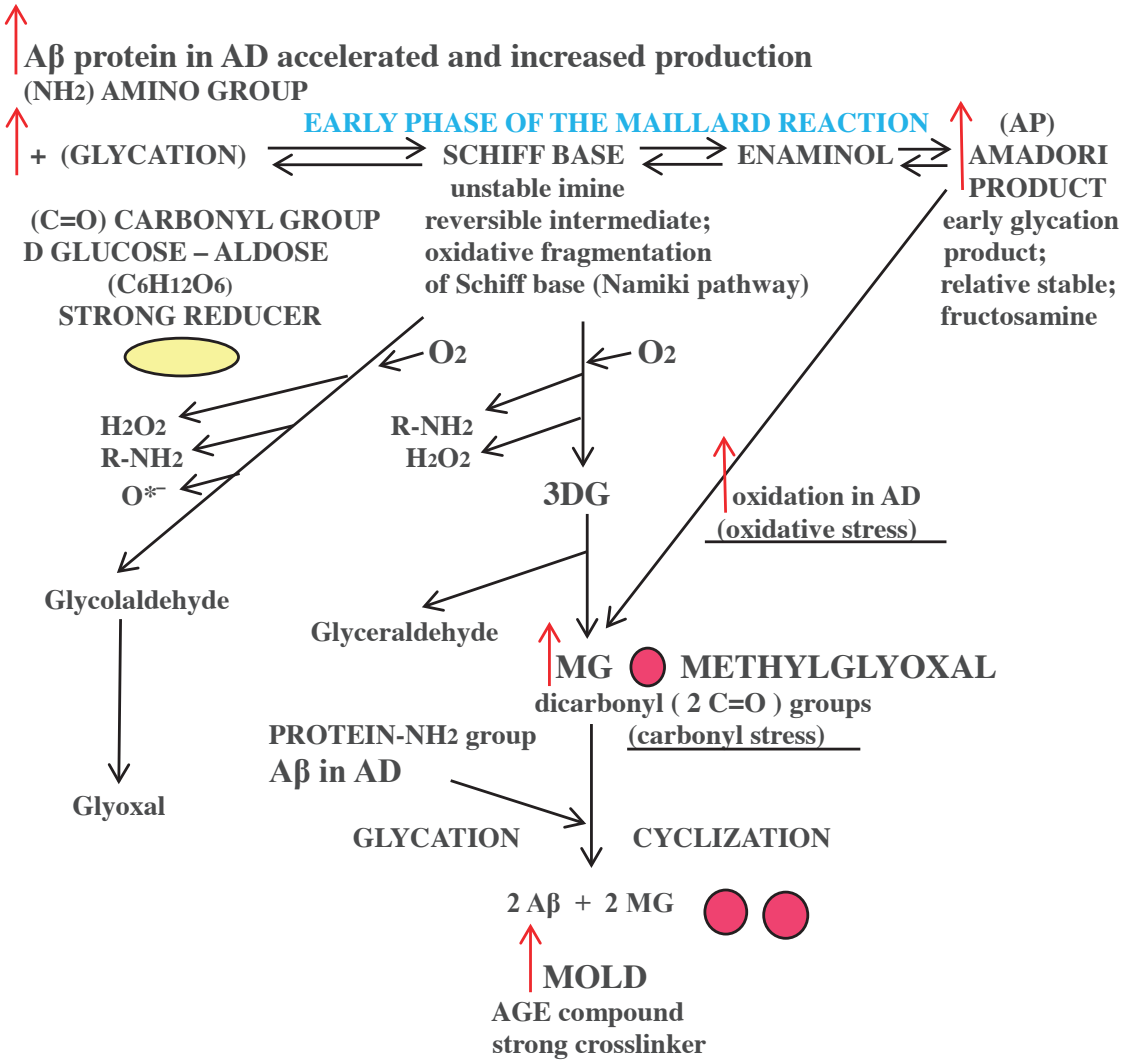

Fig. 5. **Two pathways of MG formation (Namiki pathway and AP oxidation).**

AD, Alzheimer's disease; Aβ, amyloid beta; AP, Amadori product; MG, methylglyoxal; 3DG, 3-deoxyglucosone; MOLD, methylglyoxal lysine dimer; AGE, advanced glycation end product; reduction, gain of electrons; oxidation, loss of electrons; glycation, reaction between amino group of proteins (NH_2) and carbonyl group of sugar (C=O); cyclization, formation of a ring in a chemical compound.

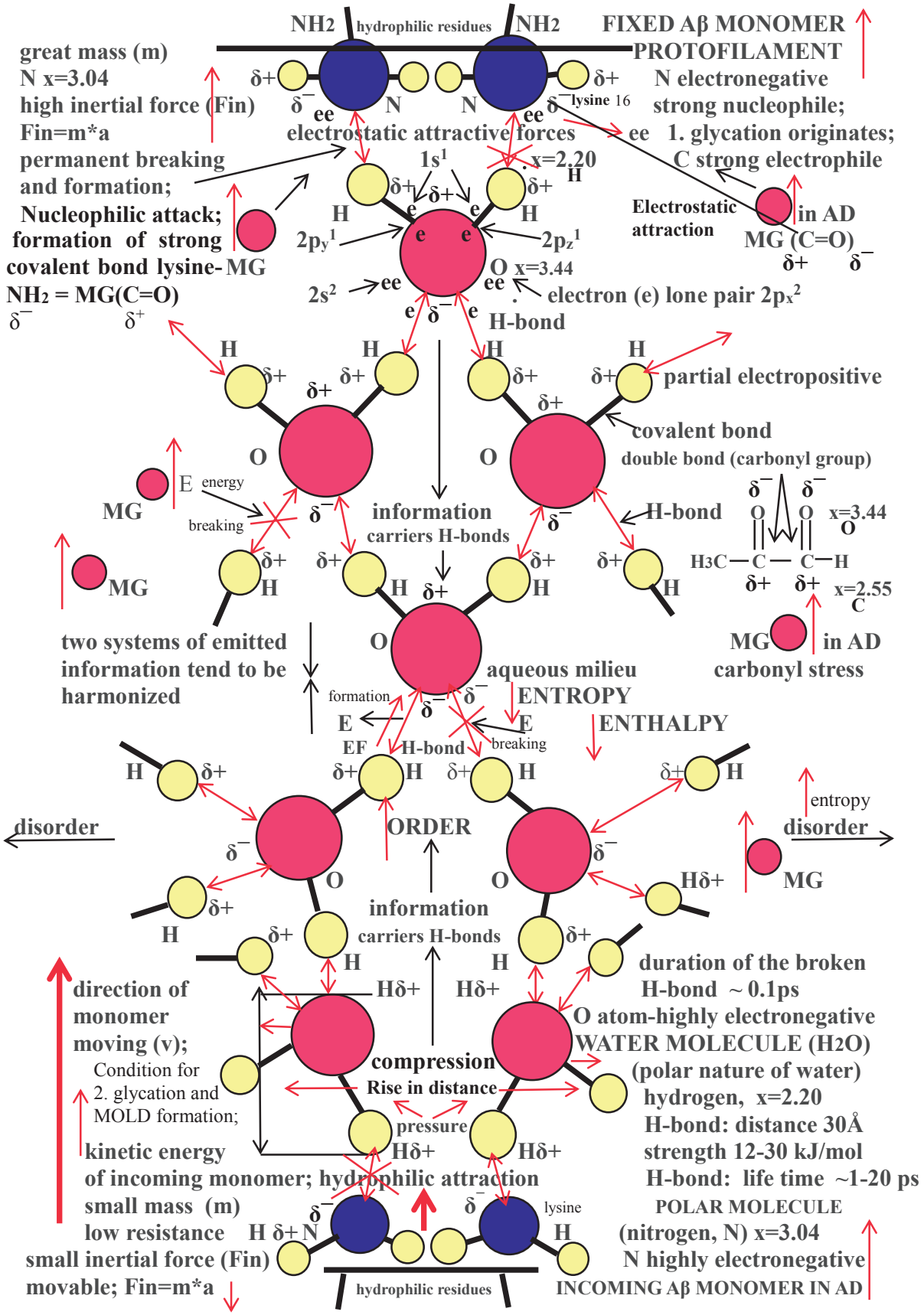

Fig. 6. **Aqueous milieu between two approaching amyloid beta monomers (attraction).**

AD, Alzheimer's disease; Aβ, amyloid beta; MG, methylglyoxal; MOLD, methylglyoxal lysine dimer; x, electronegativity; EK, kinetic energy = $m*v^2 / 2$; inertial force, Fin = m*a (accelerating force); H-bonds are permanently generated and disrupted (dynamic equilibrium); in the shell they are very strong and short – dramatic decline of the hydrogen bond dynamics near hydrophobic groups.

group of lysine and C=O group of MG (ketone group of MG) occur. Compared with the weak H-bond, the strong covalent connection (MOLD) is an important component of successful aggregation [2].

The rotation process of the incoming monomer (Aβ1) is probably also possible without the MOLD influence (center of rotation), but then the harmony between residues on the chains is slightly uncoordinated, and the rate of the reduction and aggregation is decreased. It is evident that MOLD with its effects is very harmful.

The mutual coupling of non-polar (hydrophobic, water fearing) molecules, or non-polar parts of molecules (Leu17-Ala21 on Aβ1 and Aβ2), diminishes the surfaces exposed to water, and in this way, it also reduces their destructive effect on the H-bond network (20-30 kJ/mol) between water molecules. Here lies the crucial role of van der Waals and hydrophobic interactions. The conditions for coordination between two other hydrophobic complexes, Ala30 and Leu34 on β2/β2, are now possible. The mutual removal of two redox centers rises (>19 Å) with the interruption of electron "hop" and F^{3+} reduction; the fibril extends [2,10,12].

To understand the importance of the mentioned rotation (MOLD is the center of these events), it is necessary to present a detailed explanation of the hydrophobic effect fundament, *i.e.* to explain the tendency of the non-polar, hydrophobic molecules (valine 36,18; leucine 17,34; phenylalanine 19,20; isoleucine 31,32; alanine 21; methionine 35) to mutually aggregate in the aqueous solution, removing the water. Experiments indicate that the hydrophobic attraction is not a specific force but the result of the energy required to introduce the hydrophobe into water [11,13].

During the docking process, hydrophobic residues (Leu17-Ala21 and Ala30-Leu34) permanently vibrate (vibration of amino acid residues in protein) and push the water molecules away. Congruently with the direction of the newly formed H-bonds and salt bridge forces, increasingly better contact between complementary residues finally leads to a parallel and in-register constitution.

During the oblique, off-register approach of the incoming monomer [(Aβ(1-42): length = 120 Å, 12 nm; width = 30 Å, 3 nm)] to the fixed monomer (without this event the MOLD formation is not possible), the specific pressure (crash pressure has its energy which breaks H-bonds) occurs on the water molecules in front of the incoming monomer and then these water molecules escape by breaking H-bonds. Water molecules are usually in permanent movement, consequently breaking and forming H-bonds (electrostatic attractive forces between partially negative oxygen and partially positive hydrogen – between them there is an electrostatic field – 12-30 kJ/mol) [2].

These connections are, in reality, very weak (5 kcal/mol, 12-30 kJ/mol) and can be very easily broken (the input of higher energy compared with H-bond energy is the reason of this breaking and the escaping of now free water molecules). The average duration of H-bond is 1-20 picoseconds. In one critical moment during this approaching, the MOLD (AGE) formation occurs (this is a gradual process composed of several phases: glycations, labile Schiff base formation, cyclization) and the link Lys16-Lys16 becomes stronger [2]. At the beginning, MOLD strongly supports the mentioned reduction and its harmful effects (Fenton reaction, *OH). Accompanying stronger contacts between two hydrophobic centers on β1/β1, with the incoming monomer rotation, MOLD leads to the congruity of attractive hydrophobic forces in the developed complex. Probably the congruence process develops gradually, not promptly. The mentioned rotation also induces an increasingly better contact between two hydrophobic centers on β2/β2 (the tendency for better elimination of water molecules), and monomers take up a parallel and in-register position. During these events, the interspace distance between two redox centers rises (>19 Å, hydrophobic cleft) and the reduction declines and practically disappears. The new incoming monomer better stabilizes the newly formed connection, and the mentioned process can continue [11,14]. It is evident that in this phase, MOLD supports the disruption of the reduction which is a favorable event (***Fig. 1, 2***) [2].

Special importance in these events has the development of water "cage," *i.e.* solvation shell, where the water molecules in the shell have better mutual order and restricted mobility. The molecular aggregation of both hydrophobic centers, β1/β1 and β2/β2, evidently declines the overall surface exposed to water and the consequent destructive effect on water H-bonds. In reality, this is the basis for all biological processes linked with building composed biological systems (for example protein folding). The primary reason for water "cage" generation lies in the property of non-polar molecules (hydrophobic residues on β-strands) that can break highly dynamic H-bonds between polar water molecules and develop the new composition of these links and water. The new position of the water molecules and H-bonds in the shell now has a tangential direction in relation to the hydrophobe in the center of the cage. In this way, the water "cage" is developed and is composed of water layers which are evidently more compact (the distance between water molecules is lower) than the surrounding water, and pursues non-polar molecules to aggregate into the cage. H-bonds in the water "cage" complex are evidently shorter and stronger than these bonds in the surrounding free water. Experiments indicate that the water density in the first shell near the hydrophobe is evidently higher than in the bulk. This is the basis of hydrophobic interactions. The water cage is like a protective body mechanism with the function to protect and preserve the 3D structure of surrounding water milieu. This structure has a crucial importance for the optimal proceeding of the vital biochemical reactions in the interstitium and cells (reactions that are crucial for life – high entropy, S, a measure of the disorder or randomness in a closed system). In the case of enormous destruction of 3D water structure, a catastrophe is inevitable. The water "cage" prevents this. Normally, water constitutes about 70-80% of the weight of most cells. In the absence of sufficient hydration, proteins lose their biological activity. After this explanation, it is necessary to emphasize the APP proteolysis which leads to the exit of the newly formed Aβ monomer into the local extracellular perisynaptic space. The hypothesis about the prompt interaction between two hydrophobic Aβ groups and water molecules accompanied by the 3D hydrogen bonded network disruption and formation of water "cage" around the non-polar surfaces is proved [2,12,21]. We shall see later the crucial role of water "cage" in the induction of the polarization fields and long-range attractive forces between Aβ monomers important for MOLD formation. In reality, the aggregation of hydrophobic residues is a naturally favorable process which leads to the rise of entropy in the explored system.

Permanent disruption (needs energy intake) of early formed H-bonds, and the generation of new H-bonds (release of energy), on the surfaces of both interacting monomers (the interaction between water molecules and hydrophilic residues-water loving residues), and in the interspace aqueous milieu (mutual interactions of water molecules), lead to the

instantaneous occurrence and disappearance of electrostatic fields between opposite electrified units, with the attraction of the unit with small mass and resistance (Aβ1) in the direction to the monomer on the tip of the protofilament (Aβ2). The duration of attractive forces between water molecules and hydrophilic residues is the same as the duration of the H-bond, in the range of 1-20 picoseconds. At the same time, the occurrence of more of these forces (electrostatic fields) leads to the mentioned moving of the free monomer (Aβ1). The formed trajectory induces the decline of the distance between monomers with a consequent pushing of the water molecules in all directions (***Fig. 6***). Order restoration between water molecules in the aqueous milieu interspace leads to the local drop in entropy and enthalpy (H = U + pV, H = the enthalpy of the system-heat content in a constant pressure, U = internal energy of the system, p = pressure of the system, V = volume of the system). Thermodynamics analyses by Chang and Ham [10], give strong support to the presented understanding. The formed direct contact between two monomers leads to the locking/docking events, which were explained in detail by Takeda and Klimov [9,11,12]. It is evident that the MOLD formation process significantly supports these events. On the other side, it is evident that quick direct contact between monomers also leads to quick MOLD formation.

When the incoming monomer (Aβ1) arrives to the specified distance and position (based on several investigations, approximately 30 Å, and others give evidently higher-several nm) in relation to the fixed monomer (Aβ2), the interaction of attractive forces between hydrophilic residues of two systems occurs, together with the formation of order (the direction of water dipole moments becomes dominantly uniform) in interspatial 3D water molecular structures, and attraction (the velocity-v-of movement is included) of the free monomer (Aβ1) towards the fixed monomer (attraction and final contact is the condition for MOLD formation). In relation to the fixed monomer complex, the incoming (v) monomer has evidently lower mass and inertia. At the same time, it has great kinetic energy ($Ek = m*v^2/2$) (***Fig. 6***). In their study, Chong and Ham [10] present a detailed explanation of these events. Their investigation refers to the analysis of two isolated Aβ(1-42) monomers coupling, but the results can also explain the growth of amyloid protofilament and fibril. They demonstrate that hydrophilic residues have a crucial role in these events. They emphasize that a long-range water mediated attraction of enthalpic and electrostatic origin acting on the hydrophilic residues on both monomers generates the thermodynamic force that attracts them to travel from a large separation to a contact distance [8,10]. The formed electrostatic coupling has an attractive force of 12-30 kJ/mol. It is clear that more simultaneously generated connections (energy discharge) induce the attraction of the incoming monomer towards the fixed monomer. The energy of more simultaneously formed H-bonds transforms into the kinetic energy of the incoming monomer. During these events the mentioned compression of water molecules occurs on the trajectory by pushing out from the regular direction, and thus, the disruption of 3D structures of water H-bonds appears (***Fig. 6***) [8,10].

Experiments demonstrate that hydrophilic residues of the two retired monomers through their immediate H-bonds transmit, by the next water molecules and their H-bonds in the water interspace, a lot of important information crucial for order formation and adequate contact between the two electrostatic and thermodynamic events. The consequent result is the drop of entropy and enthalpy in the interspace, the rise of kinetic energy of the incoming monomer, pushing out water molecules from the regular direction (trajectory), and the diminution of the intermonomer distance. It was found by experiments that H-bonds in the interspace form a perpendicular orientation in relation to two opposite hydrophilic groups (important for the direction of the momentary attractive electrostatic forces). If the velocity of approaching and connecting is higher, the formation of MOLD is also rapid. Vibration energy of O-H bond is also transmitted towards the contact point (CP) in the interspace. The question is how the simultaneous MG approach influences the order between water molecules (***Fig. 6***) [8,10,22-24].

By examining the origin of long range attraction between hydrophobes in water (a process crucial for MOLD formation – hydrophobic residues: Leu17-Ala21, β1 strand of Aβ and Ala30-Leu34, β2 strand of Aβ), Despa and Berry [25,26] emphasize the great importance of this attraction. In these events, the long-range electrostatic forces have a crucial role. Formation of the order of water dipoles in water "cage," with the coupling of dipole moments, induces the phenomenon of polarization field on both sides of the cavity, its strengthening, and the generation of the strong electric field between approaching monomers. Here lies the origin of the long-range attractive forces. For better understanding of these events, it is necessary to emphasize the phenomenon of permanent breaking and formation of H-bonds (their average lifetime is 10 picoseconds, 10^{-11} sec; distance ≈ 1.8 Å) between water molecules in the solvation shell. The breaking of H-bonds on the hydrophobe course enables the formation of new H-bonds between oxygen in one water molecule and hydrogen in the neighboring one. It is clear that there was a previous breaking of H-bonds between these hydrogen atoms and oxygen in the second line of the shell. Therefore, there is a breaking phenomenon in the case of tangential H-bonds (in relation to the hydrophobe) and H-bonds in the second shell's line (***Fig. 7, 8***). It is evident that there is a direct collaboration between hydrophilic and hydrophobic attractive forces. The confirmation of these findings was given by Meyer *et al.* [27]. Special importance for long-range forces between hydrophobic surfaces in hydrophobe aggregation is emphasized by Chopra and Levitt [28].

According to the author's opinion, a detailed review about the activity of hydrophilic and hydrophobic interactions is necessary for understanding the endogenous MOLD formation, its effects, and especially the oxidative stress induction (the strong Fe^{3+} reduction supporter). It is clearly evident that without these forces, it would be impossible to achieve the involved monomer contacts, based on the electrostatic attraction, as well as the endogenous MOLD formation.

Along with the great importance in the AD pathophysiology, MOLD as a strong crosslinker acts also on other tissues, especially those located in arterial walls. By crosslinking the collagen and elastic fibers, MOLD leads to the increased stiffness of walls and drop in their elasticity and compliance. The consequent rise of local blood pressure induces many pathological mechanisms which accelerate the atherosclerotic process. This is congruent with the frequent apparition of atherosclerotic damages connected with AD [2,29].

The presented study undoubtedly indicates the great importance of MG and AGE compound MOLD generation. MG can also possess the exogenous origin. The important role for this is the use of specific food. In their detailed study, Uribarri *et al.* [30] present the chemical and nutritional analysis of usual food with its exact AGE content. This is especially

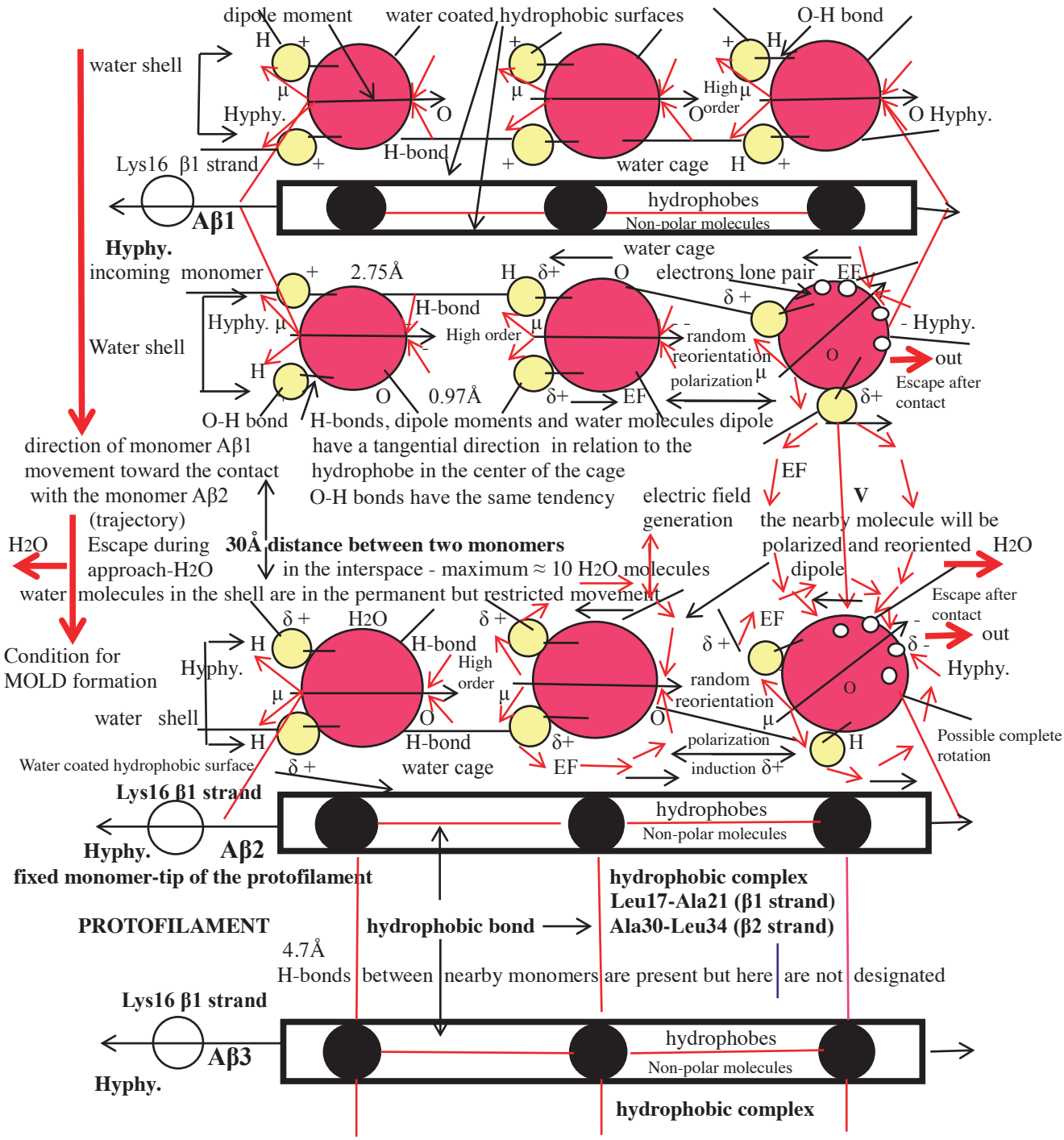

Fig. 7. **Schematic presentation of the water cage shell and initial formation of electrostatic field between two separated monomers – condition for MOLD formation.**

Between stacked Aβ monomers in the protofilament, as well as in the hydrophobic cleft, there are no water molecules; Hyphy, hydrophyles; there is a visible random reorientation of two opposite water dipoles which make the opportunity for electrostatic field generation; the next phase is the polarization of the adjacent dipoles, their rotation, and polarization field formation. Between two opposite polarization fields occurs the strong electrostatic attraction; H, hydrogen; O, oxygen; μ, dipole moment; protofilament, structure composed of a number of stacked Aβ monomers; in the chemistry convention the arrow representing the dipole moment points from positive to negative – in physics, the opposite is used; Aβ, amyloid beta; MOLD, methylglyoxal lysine dimer; EF, electric field; V, potential energy for attraction, $V = -\mu_1 \mu_2 / 4 \pi \varepsilon_0 r_{12}^{3*} (\cos O_{12} - 3\cos O_1 \cos O_2)$, μ, dipole moment; r, distance between two dipoles; ε_O, space permeability; $\cos O_{12}$, angle between two opposite dipoles; $\cos O_1$ and $\cos O_2$, angles formed by two dipoles with respect to connecting lines.

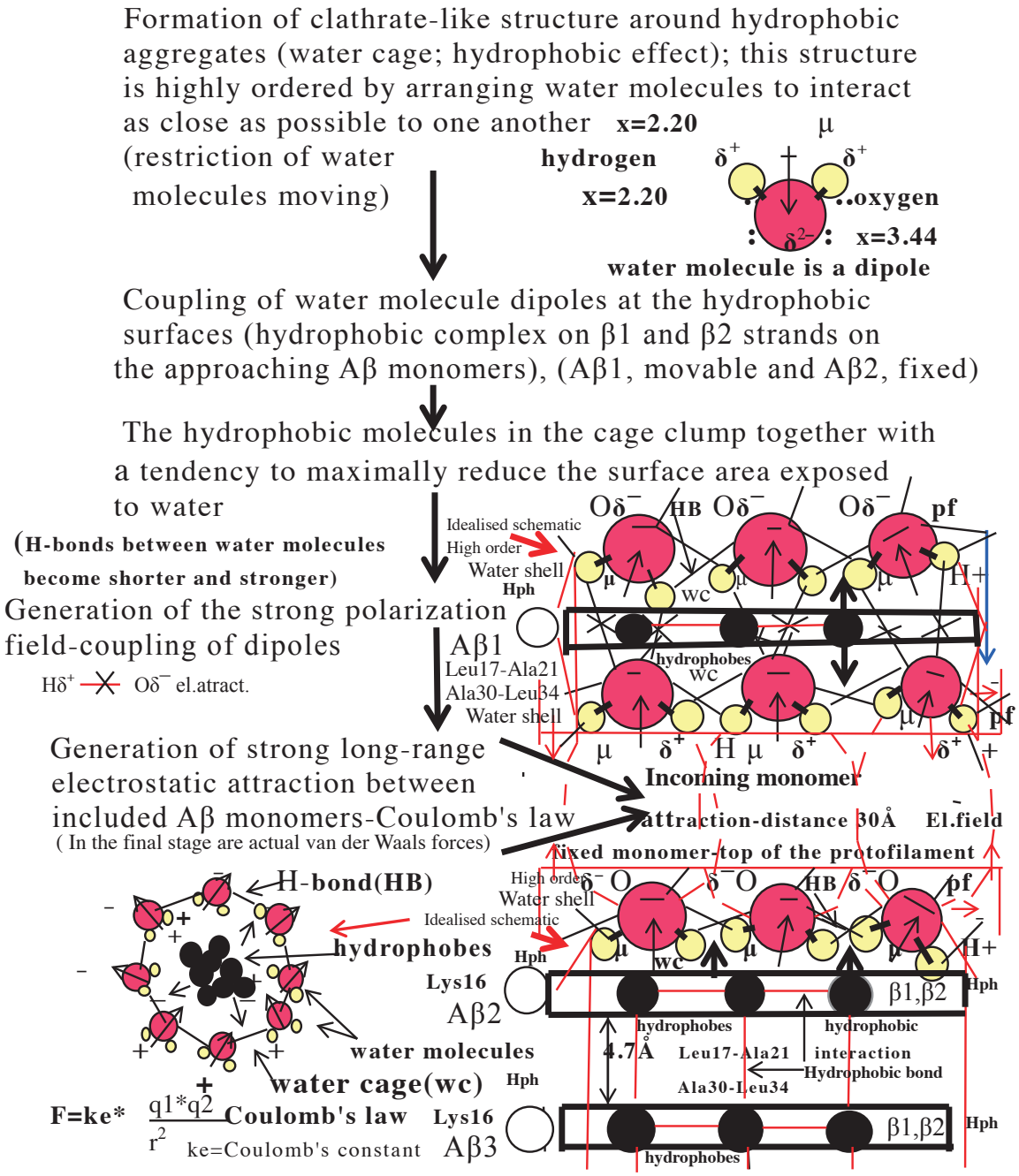

Fig. 8. **Formation of water cage, polarization field (pf) and long-range electrostatic attraction.**

The figure represents the situation after the reinstatement of Aβ1/Aβ2 electric field. μ, dipole moment vector, dipole moment is a measure of the polarity of the molecule; Aβ, amyloid beta; pf, polarization field; x, electronegativity; δ^-, partial negative charge; δ^+, partial positive charge; dipole, one end of the molecule has a higher concentration of electrons than the other end; convention: in chemistry the arrow representing the dipole moment has a direction from positive to negative end – in physics, this is opposite; wc, water cage; Hph, hydrophilic; F = force of the interaction; q1 and q2, magnitude of the charges; r, distance between charges; after the realized Aβ1/Aβ2 contact the water molecules from both sides push out from the contact site; presented figure actually describes the situation after the complete polarization has been established.

related to the method of preparing different food, the way of cooking and exposure to high temperatures during cooking. AGE compounds that are recorded into the body, through food, lead to oxidation stress, the crucial factor in the occurrence of diabetes mellitus, cardiovascular, neurodegenerative diseases, and the aging process. They especially point out the harmful effects of foods rich in proteins and lipids. The entrance of AGEs into the body evidently declines by the use of food rich in carbohydrates, such as fruit, vegetables, cereals and milk. They especially point out the importance of food preparation on lower temperatures, on steam, or by using the preliminary marination (in vinegar, or lemon juice). It is possible to slow down the MOLD generation and to strengthen its breaking by the same compounds, among them they especially emphasize alagebrium (ALT-711; DMPTB [N-phenacyl-4,5-dimethylthiazolium bromide], the breaker of already-formed crosslinked compounds); aminoguanidine (CH6N4, pimagedine – it suppresses the crosslinking process, nitric oxide synthase inhibitor); and DPTC (4,5-dimethyl-3-phenacylthiazolium chloride), which is also a breaker of the already formed crosslinked compounds [1,30-34]. To reverse the MG entrance in the body, it is necessary to reduce the intake of coffee, toast, cigarette smoking and soy sauce. *Ginkgo biloba* is under intensive investigations – especially its neuroprotective and anti-apoptotic capacity [35]. They found its strong anti-oxidative effect – the blockade of membrane lipid peroxidation. There are many other vitamins with antioxidant capacity, especially vitamin C, α-tocopherol, thiamine and pyridoxamine [1,36-39]. However, the subject of this study is not a detailed review of this issue. It is necessary to mention the recently actual medicamentous AD therapy. In fact, there are two groups of medicaments. The first group consists of cholinesterase blockers: Aricept (donepezil hydrochloride) [44], Exelon (rivastigmine), and Reminyl (galantamine hydrobromide). The second group includes N-methyl-D-aspartate (NMDA) receptors (NMDARs) antagonists, as well as Ebixa (memantine) [1].

Conclusion

AD is a wasting, chronic, and, until now, still incurable neurodegenerative disease. The exact reason for this disease has yet to be completely discovered, but many data indicate its polygenetic etiology. In its origin and course, there is undoubtedly the great importance of AGEs, especially of MOLD and its precursor MG. Generated through the composed glycation processes, by triose phosphate pathway, the Namiki pathway, and Amadori product oxidation, through a number of biochemical reactions, especially cyclization, MG generates the strong cross-linking factor MOLD, which is crucial for the amyloid beta molecule aggregation and the reduction of non-toxic redox inactive Fe^{3+} in toxic redox-active Fe^{2+}. In these processes, which lie in the basis of AD, are tightly intertwined electrostatic, hydrophobic and hydrophilic forces. Their analysis is recently in the intensive phase of investigation. Undoubtedly, the explanation of their interaction will contribute to AD effective therapy. The presented study is a small contribution to providing a solution for this great medical and social problem.

Conflict of Interest Statement

Part of this research was presented at the 27th Summer Stroke School "Healthy Lifestyle and Prevention of Stroke" on June 9, 2016, in Dubrovnik, Croatia.

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The author states that performance of this study entailed no issues representing a conflict of interest.

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