

Review

# Glycation—a sweet tempter for neuronal death

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## Abstract

Glycation, one of the post-translational modifications of proteins, is a nonenzymatic reaction initiated by the primary addition of a sugar aldehyde or ketone to the amino groups of proteins. In the early stage of glycation, the synthesis of intermediates leading to the formation of Amadori compounds occurs. In the late stage, advanced glycation end products (AGE) are irreversibly formed after a complex cascade of reactions. Several AGEs have been characterized chemically, while other new compounds remain to be identified. To date, studies of the contribution of glycation to diseases have been primarily focused on its relationship to diabetes and diabetes-related complications. However, glucose-induced damage is not limited to diabetic patients. Although it does not cause rapid or remarkable cell damage, glycation advances slowly and accompanies every fundamental process of cellular metabolism. It has recently become clear that glycation also affects physiological aging and neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis. Glycation alters the biological activity of proteins and their degradation processes. Protein cross-linking by AGE results in the formation of detergent-insoluble and protease-resistant aggregates. Such aggregates may interfere with both axonal transport and intracellular protein traffic in neurons. In addition, glycation reactions lead to the production of reactive oxygen species. Conversely, glycation is promoted by oxidative stress. We speculate on the presence of synergism between glycation and oxidative stress. In this review, we provide an outline of glycation and propose some possible mechanisms of its cytotoxicity and defense systems against it.

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## 1. Introduction

Glycation, also called the Maillard reaction, is a nonenzymatic reaction initiated by the primary addition of a sugar aldehyde or ketone mainly to the  $\epsilon$ -amino group of lysine residues and the  $\alpha$ -amino group at the N-terminus of proteins (Fig. 1) [29,82]. The synthesis of intermediates leading to the formation of Amadori compounds occurs in the early stage of glycation. In the late stage, advanced glycation end products (AGEs) are irreversibly formed after a complex cascade of repeated dehydration, condensation, fragmentation, oxidation, and cyclization reactions (Fig. 2). Chemically characterized major AGE are:  $N^{\epsilon}$ -(carboxymethyl)lysine (CML) [18], pentosidine [16], pyrroline [57], and imidazolone [63] (Fig. 3). Glycation alters the biological activities of proteins [3,6,25] and their degradation processes. Protein cross-linking by AGE results in the formation of detergent-insoluble and protease-resistant aggregates [95]. Such aggregates may interfere with both axonal transport and intracellular protein traffic in neurons by physical blockage (Fig. 4).

To date, studies of the contribution of glycation to diseases have been primarily focused on its relationship to diabetes and diabetes-related complications [61,77,115]. However, glucose-induced damage is, of course, not limited to diabetic patients: even at normal glucose levels some degree of glycation occurs, and the resulting damage will accumulate over time. It has become clear that glycation and AGE also affect physiological aging (senile

cataracts, arteriosclerosis, etc.) [113] and neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease [10], and amyotrophic lateral sclerosis (ALS) [15,88].

From 1994 to 1995, a series of reports appeared on glycation as an important factor in the pathomechanism of AD. Opposing opinions were also presented, and the discussion has persisted as to whether glycation is a primary cause. In this regard, we recently demonstrated the existence of an Amadori product, an early compound in the glycation reaction, in the ALS spinal cord [39]. A number of other reports indicate an association of glycation with ALS [15,88]. In addition, the glycation reaction leads to the production of reactive oxygen species. A large amount of reactive oxygen species is produced through the development of glycation [29,36,59,78,97,119]. Conversely, glycation is promoted by oxidative stress, which seems to be a leading causative factor in cell damage in aging and in neurodegenerative diseases. The involvement of reactive oxygen species in the cytotoxicity of the glycation reaction was demonstrated in our experiment with cultured cerebral cortical and spinal neurons. These observations led to the hypothesis that glycation-induced pathological conditions result from a series of oxidative stresses, increased modification of proteins via the glycation reaction, and further AGE-dependent oxidative stress [93].

Glycation does not cause rapid or remarkable cell damage since it advances slowly. Nevertheless, the significance of glycation should not be ignored because it

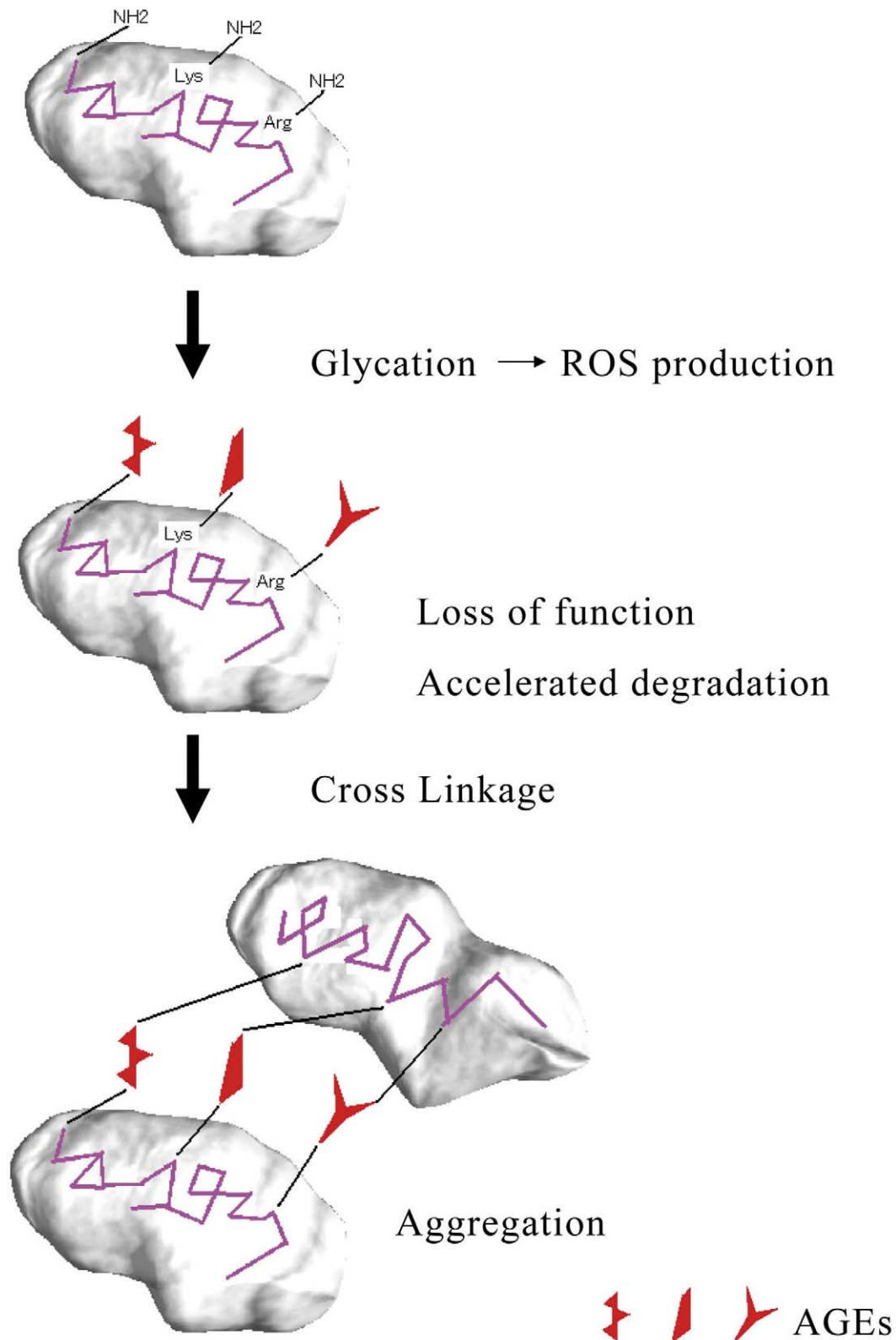


Fig. 1. Advanced glycation end-product (AGE) formation and its effect on proteins. A nonenzymatic reaction between glucose or other reducing sugar and the N-terminal amino acid residues and/or  $\epsilon$ -amino groups of proteins initially forms a Schiff base adduct. The Schiff base adduct then slowly undergoes Amadori rearrangement. Additional dehydration, condensation, fragmentation, rearrangement, and oxidation reactions occur, which ultimately create a structurally heterogeneous group of adducts that remain bound irreversibly to the protein. Reactive oxygen species (ROS) are produced via a glycation reaction. AGE proteins lose their specific functions and undergo accelerated degradation, while AGE can also act as a cross-linker between proteins, resulting in proteinase-resistant aggregates.

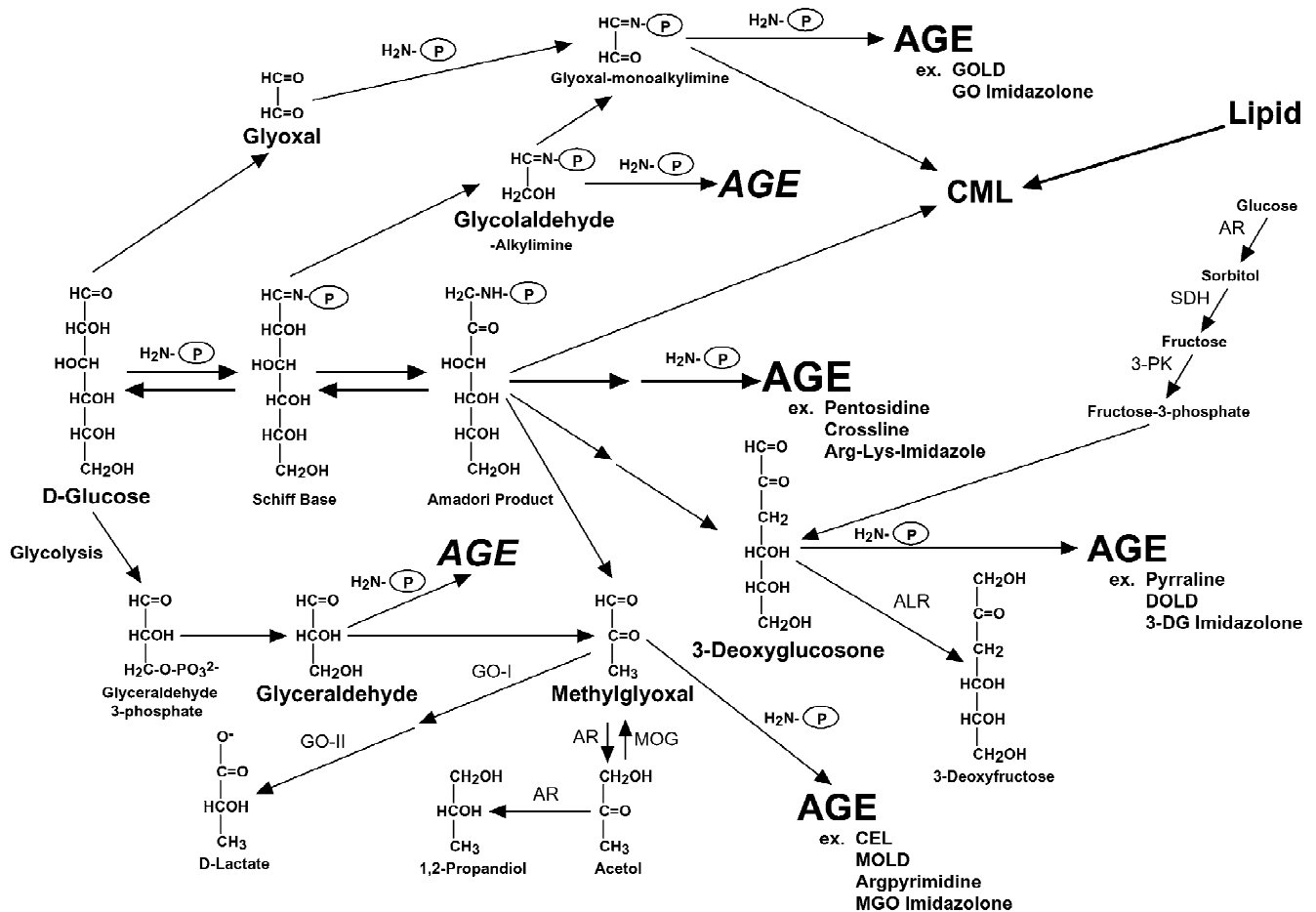


Fig. 2. Possible routes of AGE formation. AGE arise from decomposition of Amadori products, a glycolysis intermediate product (glyceraldehyde), a Schiff base fragmentation product (glycolaldehyde), and Amadori product fragmentation products (methylglyoxal and 3-deoxyglucosone), and the autoxidation of glucose to glyoxal. GO imidazolone, glyoxal imidazolone; MGO imidazolone, methylglyoxal imidazolone.

accompanies every fundamental process of cellular metabolism.

In this review, we provide an outline of glycation and propose some possible mechanisms of its cytotoxicity and defense systems against it. We give an overview of the role of glycation in the pathomechanism of AD, and then discuss the synergism between glycation and oxidative stress on the basis of the results of our *in vitro* study using cultured cerebral cortical neurons. Finally, we review the evidence of glycation in ALS spinal cords, and then discuss its neurotoxicity on cultured spinal motor neurons.

## 2. Post-translational modifications of proteins and neurodegenerative diseases

With regard to the etiology of familial neurodegenerative diseases, the identification of causative genes producing abnormal proteins has been actively investigated. For all but a few conditions, however, the mechanisms by which such abnormal proteins lead to the onset of diseases

are largely unknown. Complicating the investigation is the fact that most cases of neurodegenerative disease are sporadic without any family history. Aberrant turnover with abnormal post-translational modifications of proteins may be involved in the pathomechanisms of neurodegenerative diseases, with or without abnormal genes. Most neurodegenerative diseases have multiple, i.e. genetic and environmental, causes, and aging is an important common factor in the onset and progression of disease. Neurodegenerative diseases will be major targets for 'post-genome era' research.

Accumulated modifications in proteins may be related to the onset and progression of disease. What sort of modifications occur in proteins? Known post-translational modifications of proteins include phosphorylation, oxidation, nitration, ubiquitination, glycation, racemization, and isomerization, to name but a few. Glycation promotes inter- and intraprotein linkages, accompanied by changes in biological activity, and results in aggregate formation. Furthermore, reactive oxygen species arising during glycation would also be likely participants in cell damage.

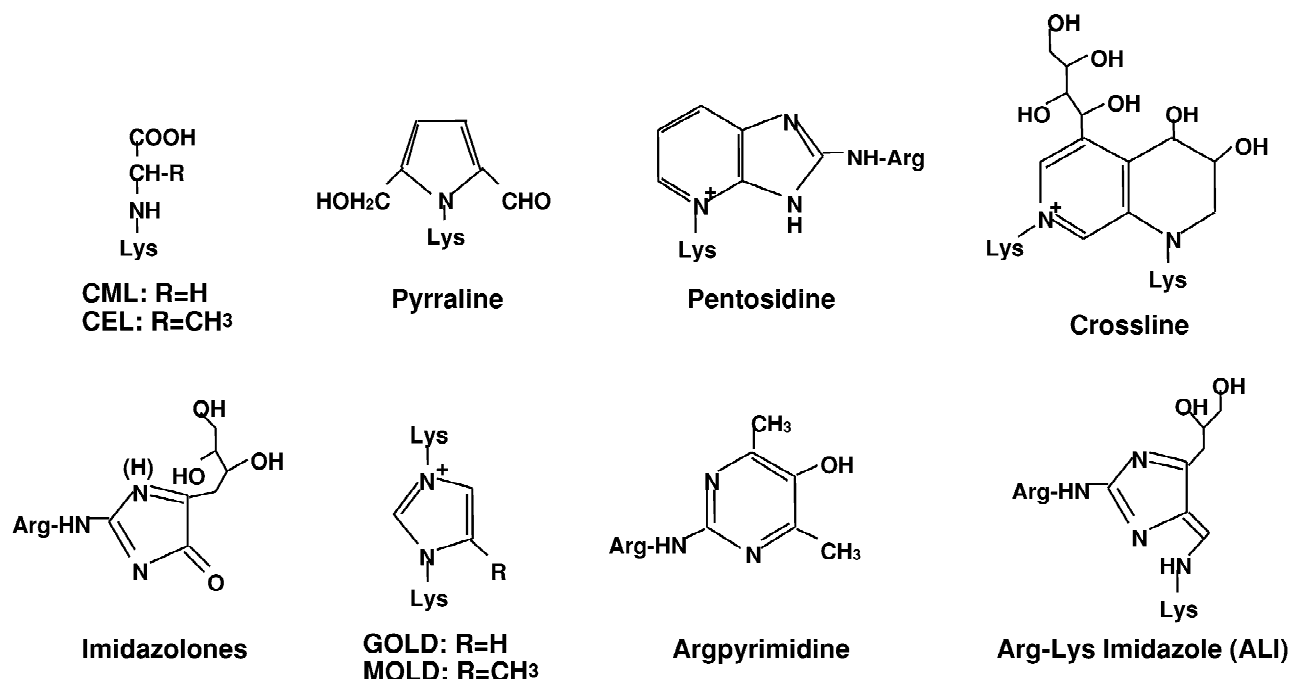


Fig. 3. Chemical structures of various AGE: CML,  $N^{\epsilon}$ -(carboxymethyl)lysine; CEL,  $N^{\epsilon}$ -(carboxyethyl)lysine; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer.

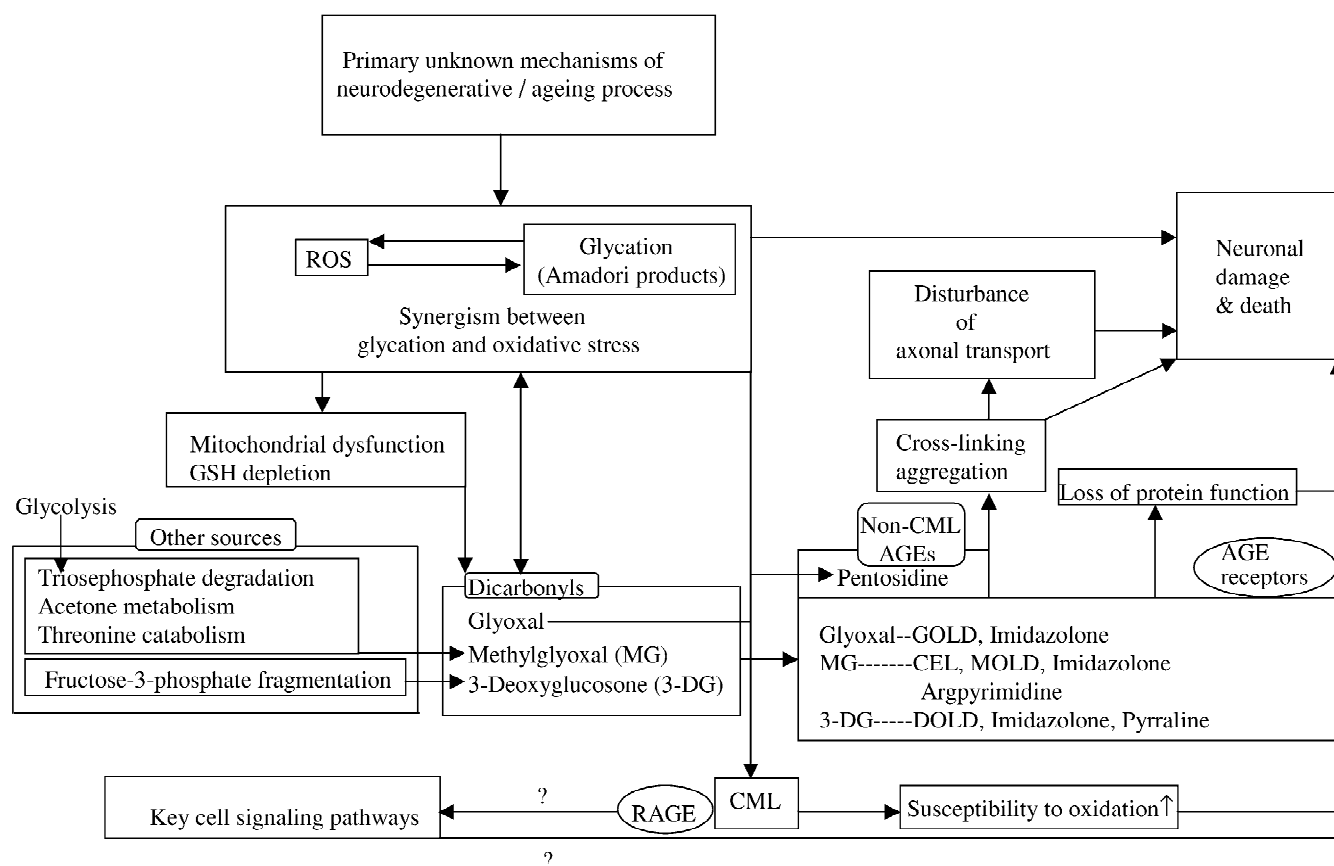


Fig. 4. Glycation and AGE in the process of neuronal death. ROS, reactive oxygen species; NO, nitric oxide; RNOS, reactive NO species; GSH, reduced glutathione; DOLD, 3-DG-lysine dimer; RAGE, receptor for AGE.

### 3. Glycation

Research on glycation began with the discovery by Louis Camil Maillard that heating amino acids and reducing sugars together results in a color change to yellowish brown [55].

The enzymatic addition of sugars to proteins is called glycosylation, while the nonenzymatic addition of reducing sugars is called glycation. However, these terms are not strictly differentiated and glycosylation is sometimes used to refer to both the enzymatic and nonenzymatic addition of sugars. Glycation is also referred to as the ‘Maillard reaction’ in honor of Louis Camil Maillard.

Glycation can be divided into two reaction stages, namely an early-stage reaction and a late-stage reaction (Fig. 2). The early-stage reaction starts with the interaction of reducing sugars, such as glucose, with free amino groups on the N-terminus, lysine residues, and arginine residues in proteins. Via a Schiff base, a relatively stable Amadori product is synthesized. The reaction is reversible up to the point of the synthesis of the Amadori product and is dependent on compound concentrations and incubation time. If lysine residues are present on the molecular surface, glycation occurs readily, and is facilitated by the presence of a histidine residue near the lysine [3]. The late-stage reaction is an irreversible reaction, involving dehydration, hydrolysis, etc., and consequently results in the formation of AGE.

### 4. Generation and structure of AGE

In the late stage of glycation, AGE are formed after a complex cascade of repeated dehydration, condensation, fragmentation, oxidation, and cyclization reactions via intermediates such as 3-deoxyglucosone (3-DG) (Fig. 2). The late-stage reactions are irreversible. Structure-identified AGE include *N*<sup>ε</sup>-(carboxymethyl)lysine (CML) [18], pentosidine [16,23], pyrraline [57], imidazolone [63], crosslin [67], etc., while other new compounds have yet to be identified (Fig. 3).

CML is formed from fructose lysine (one of the Amadori products) in the presence of metal ions. In addition to glucose, other sources of CML include unsaturated fatty acids such as oleic acid and linoleic acid. The idea that CML is a marker of oxidation rather than of glycation is a recent trend [18].

Pentosidine is formed from lysine and arginine with either glucose, fructose, ribose, ascorbic acid, or 3-DG [16,23]. Pentosidine increases in skin collagen and lens crystallin rectilinearly with age. The amount of pentosidine doubles in the plasma of diabetic patients [16].

Pyrraline is formed from 3-DG and lysine, and doubles in diabetic patients [57]. Imidazolone is formed from 3-DG and arginine [64]. Crosslin is produced from two molecules of glucose and two lysines [67].

For the cytotoxicity of AGE, intermediates of glycation with two reactive groups such as dicarbonyl compounds are of great importance (Fig. 4). These intermediates act as cross-linkers. CML and pyrraline, in contrast, do not provide cross-linkage. Protein cross-linkage results in aggregates, which then form intracellular protease-resistant and ubiquitin-proteasome-resistant deposits, consequently inhibiting the intracellular transport of materials. Given the importance of axonal transport in the maintenance of neurites and the supply of neurotrophic factors, interference with axonal transport would cause serious problems.

### 5. Inhibition of basic protein functions by glycation

The cytotoxicity of glycation results from the following three mechanisms (Fig. 4): (1) inhibition of specific functions of proteins; (2) cross-linkage, aggregation, and precipitation of proteins; and (3) production of reactive oxygen species. Neurons are cells that have stopped dividing and can no longer separate out or dispose of metabolic waste products, and contain several proteins with a slow rate of turnover. Consequently, it is considered that the cytotoxicity of glycation in neurons always progresses slowly and latently. Through glycation, specific functions of protein are altered, i.e. the function is lost or its level is reduced. In addition to functional changes at active sites, changes in functions are also caused by accelerated or delayed degradation of glycated proteins.

The effects of glycation on some representative cytoskeletal proteins and enzymes in neurons are discussed in detail in the following paragraphs. Neurofilaments are neuron-specific, medium-sized fibers [32]. A common pathological finding in ALS is the presence of an abnormal accumulation of neurofilaments (hyaline inclusions and axonal spheroids) in the cell body and proximal axon of surviving motor neurons. Neurofilaments have three isoforms, which are referred to as light (NF-L), medium (NF-M), and heavy chains (NF-H). The tail domain of NF-H has multiple repeats of Lys–Ser–Pro (KSP), accounting for the unusually large presence of phosphoserine residues in this protein. Each phosphorylation site is neighbored by lysine residues, which are potential glycation sites. In addition, neurofilaments have a long half-life. The glycation of neurofilaments was first reported in peripheral nerves in diabetes mellitus (DM) and in brain cytoskeletal proteins [8]. Recently, Chou et al. proposed that glycation of neurofilaments plays a fundamental role in motor neuron diseases [14].

Tau is a microtubule-associated protein. Inclusion bodies and intracellular deposits associated with tau are implicated in AD and other neurodegenerative diseases. The glycation of tau has also been extensively studied with regard to AD [49]. Glycation was probed in soluble and insoluble PHF (paired helical filaments)-tau from AD brains using anti-CML antibody. The results demonstrated

that tau becomes glycosylated in PHF-tau and that the glycosylation may play a role in stabilizing PHF aggregation, which leads to tangle formation in AD [45]. Glycosylated tau exhibited a loss of capacity in the promotion of microtubule assembly in vitro [21].

Since mutations of the superoxide dismutase-1 (SOD-1) gene were first identified in 1993, it has been considered a possible cause of familial ALS (FALS) [76]. Bredesen advocated the possibility that SOD-1 even plays a role in sporadic ALS [7]. The glycosylation of SOD-1 under diabetic conditions has been studied extensively by Taniguchi et al. [3], who found that enzymatic activity was attenuated by glycosylation both in vivo and in vitro. Glycosylation prompts the degradation of SOD-1 [70]. In the lens of diabetic rats, glycosylation and the degradation of SOD-1 were clearly recognized [104]. Mutant SOD-1 is more easily glycosylated than normal SOD-1 and would therefore be more rapidly degraded. In addition, mutant SOD-1 has a low affinity for copper. Copper released from mutant or glycosylated SOD-1 would promote the generation of hydroxyl radicals by the Fenton reaction.

## 6. Receptor for AGE (RAGE) and AGE-binding proteins

RAGE was cloned as a receptor for removing AGE. Scavenger receptors (SRs) are also responsible for removing AGE on macrophages. Although many are as yet unknown with regard to receptor function, three proteins have been reported as AGE-binding proteins: AGE-R1 (oligosaccharyl transferase complex protein 48 (OST48)), AGE-R2 (80K-H protein), and AGE-R3 (galectin-3).

Yan et al. demonstrated that RAGE and SR mediate cell adhesion to amyloid  $\beta$  protein (A $\beta$ ) and induction of oxidative stress [118]. El Khoury et al. (1996) also showed that SR mediates adhesion of microglia to A $\beta$  fibrils and leads to the generation of reactive oxygen species and cell immobilization [17].

RAGE is a member of the immunoglobulin superfamily of cell-surface molecules, and is expressed in a variety of cell types, including endothelial cells, smooth muscle cells, mononuclear phagocytes, and neurons. SR is a homotrimeric cell-surface molecule and is expressed only on resident macrophages or microglial cells. Both receptor types are up-regulated in affected regions in an AD brain and are characterized by having a high affinity and broad specificity for ligand binding; both bind AGE. RAGE and SR are involved in signaling microglia to accumulate at sites of A $\beta$  deposition. A $\beta$  induces the production of tumor necrosis factor- $\alpha$  by microglia.

Signal transduction systems after RAGE production have recently been investigated [30,44]. Chou et al. demonstrated the presence of R1=OST-48 on neurofilament conglomerates in primary motor neurons in the motor cortex and spinal motor neurons [13].

## 7. Glycation in Alzheimer's disease

Recently, three reports were published concerning glycation in AD [11,80,93]. To best evaluate the relevance of these three reports, some previous reports are reviewed below.

### 7.1. Immunohistochemical and biochemical studies on glycation in AD

The possibility of the involvement of glycation in AD was first suggested in several reports published successively during 1994–5 [95,112,119,120]. Senile plaques and neurofibrillary tangles were positively stained with anti-pyrraline and anti-pentosidine antibodies [95]. Münch et al. reported that the accumulation of intracellular AGE was observed in up to 95% of pyramidal neurons in patients with presenilin-1 mutations [60]. AGE coexisted with hemoxygenase-1 (HO-1) on neurofibrillary tangles [119]. Since HO-1 is induced under oxidative stress, it was speculated that reactive oxygen species were produced by tau modified with AGE leading to the induction of HO-1. AGE increased three-fold in AD brains.

### 7.2. In vitro studies

In vitro glycation of A $\beta$ , tau, and apoprotein E (apoE), and their effects were investigated. The seeding effect of A $\beta$  increased after the modification of AGE [112]. In addition to hyperphosphorylation, the glycation of tau leads to the formation of paired helical filaments in AD [48]. The glycation of apoE impairs its binding to heparin [93]. AGE-modified tau produced reactive oxygen species in cultured cells and also caused an increase in the production and secretion of A $\beta$  [120]. Yan et al. provide compelling evidence that A $\beta$  interacts with RAGE in neurons, microglia, and vascular endothelial cells. Their data also demonstrate that this interaction mediates cell adhesion to A $\beta$  and the induction of oxidative stress in microglia [118].

### 7.3. Pros and cons for glycation in Alzheimer's disease

Harrington and Colaco reviewed studies of glycation in AD and proposed support for the involvement of glycation in AD [24]. Smith et al. suggested that age-related increases in oxidative stress and protein glycation either individually, or more likely in a synergistic manner, could, exclusive of other theories or in concert with them, account for all aspects of AD [97].

At the same time, an opposing opinion was expressed concerning the involvement of glycation in AD [56]. These authors indicated that A $\beta$  can form aggregates and produce reactive oxygen species (ROS) even if it is not modified by AGE. AGE do not occur in immature plaques and neurofibrillary tangles, the main criticism Mattson cited in rejecting the significance of glycation in AD. Nevertheless,

he did not deny that glycation may play a role in AD as a disease accelerator or modifier. Indeed, he wrote an editorial in favor of the study of RAGE and A $\beta$  by Yan et al. [56].

#### 7.4. Glycation in astrocytes, not in senile plaques or NFT

Soon thereafter, several reports rejecting the idea of the direct contribution of glycation in senile plaques and neurofibrillary tangles to AD pathomechanisms were presented consecutively. These reports discussed the significance of glycation in AD in ways other than in the formation of senile plaques and neurofibrillary tangles.

Before referring to those reports, the relationship between aging and the accumulation of AGE should first be reviewed, since they are both closely related. CML is reported to increase with aging in the pyramidal neurons of the hippocampus [42]. The same authors demonstrated that antibodies against AGE such as pentosidine and FFI stained lipofuscin, corpora amylacea, axonal dystrophy, and Marinesco bodies [43]. These structures are often associated with aging. One report described how the pyramidal neurons were stained selectively with an anti-AGE-poly-L-lysine antibody, suggesting that AGE is a contributor to neuronal aging [50].

In AD brains, the presence of CML was confirmed both inside and outside the cytoplasm of neurons. However, CML is also present in the cytoplasm of neurons of healthy old people [105]. In addition, the CML outside the cytoplasm of neurons was not related to senile plaques. AGE (pentosidine and CML) are co-localized with lipofuscin outside the cytoplasm of neurons [26]. In many cases, lipofuscin is produced by lipid peroxidation. The presence of extraneuroprikaryal pentosidine and CML was finally confirmed in astrocytes and microglia, and this was proven specific to AD pathology [105,106]. CML and pentosidine have recently become to be considered as markers of oxidative stress rather than of glycation.

Shibata et al. showed that A $\beta$ -, AGE-, and RAGE-positive granules were identified in the perikaryon of hippocampal neurons (especially forms CA3 and CA4) in all subjects (AD and diabetes mellitus (DM) patients). In AD patients, most astrocytes contained both AGE- and RAGE-positive granules and their distribution was almost the same. In DM patients and controls, the presence of AGE- and RAGE-positive astrocytes was very rare. These findings support the hypothesis that glycated A $\beta$  is taken up via RAGE and is degraded via the lysosomal pathway in astrocytes. AGE-immunopositive granules were observed in AD, DM, and control brains. Many AGE-positive granules were identified in the perikaryon of neurons, and this staining pattern did not exhibit any remarkable differences between the groups. The presence of AGE in neurons was thought to reflect aging. AGE-positive granules in neurons have been reported to accumulate in an

age-dependent manner. Therefore, AGE-positive granules in neurons may not have a pathological meaning, and are more likely related to the normal aging process. In AD brains, most astrocytes (approximately 70–80%) contained both AGE- and RAGE-positive granules, and their distribution was almost the same, while fewer astrocytes contained A $\beta$ -positive granules (approximately 20–30%). This finding indicates the presence of glycated proteins other than A $\beta$ . In the onset and progression of AD, the presence of AGE- and RAGE-positive astrocytes is thought to be more significant than the presence of only AGE.

Wong et al. reported that AGE-positive astrocytes and microglia expressing iNOS were found in AD brains, and concluded that the activation of microglia and astrocytes by AGEs with oxidative stress may be an important factor in AD [116].

Those studies emphasized the significance of astrocytes and microglia, but did not answer questions concerning the role of glycation of senile plaques and neurofibrillary tangles in the pathomechanism of AD.

#### 7.5. Glycation in early plaques

It was reported that senile plaques, even diffuse or primitive ones, were positively stained by a certain antibody against AGE [79]. This antibody does not recognize pyrrolidine, pentosidine, CML, FFP (2-furoyl-4[5]-[2-furanyl]-1-*H*-imidazole), or AFGP (1-alkyl-2-formyl-3,4-diglycosyl-pyrroles). These findings support the possibility that glycation is involved in the initial stage of plaque formation. Neurofibrillary tangles were also stained positively in this study.

#### 7.6. Active glycation in neurofibrillary pathology

The study by Castellani et al. [11] concerned *in situ* techniques to assess CML, the predominant advanced glycation end-product that accumulates *in vivo*, along with its glycation-specific precursor hexitol-lysine, in patients with AD as well as in young and in aged-matched controls. The levels of both CML and hexitol-lysine increased in the neurons, especially in those cases exhibiting intracellular neurofibrillary pathology. Their findings of concurrent increases in both the end-stage modification (CML) and the initial Amadori products of sugar adduction provide evidence for an active glycation process in AD. In addition, because CML can result from either lipid peroxidation or advanced glycation, whereas hexitol-lysine is solely a product of glycation, this study, together with studies demonstrating the presence of 4-hydroxy-2-nonenal (HNE) adducts and pentosidine, provides evidence of two distinct processes acting in concert in AD neuropathology. It is suggested that AGE are not only a critical factor leading to the formation of hallmark inclusions, but are also part of the early changes seen in the disease process.



In this study, most of the amyloid core of a subset of senile plaques showed no staining or only faint immunoreactivity for CML. Variable vascular immunoreactivity was noted in both AD cases and in the controls with antibodies to CML and hexitol-lysine. Glial cells remained unstained.

Neurons containing neurofibrillary tangles incidentally found in a control subject also showed strong labeling with the antibodies to CML and hexitol-lysine, supporting the hypothesis that glycation is an early event, preceding inclusion formation in AD.

#### 7.7. Conclusion about the immunohistochemical studies

Castellani et al. stated their opinions on the discrepancies existing among reports on glycation in AD [11]. In other studies, the distribution of neuronal and glial deposits of CML did not correspond with the distribution of AD pathology, causing those authors to conclude that CML does not directly cause the formation of neurofibrillary tangles or neuronal loss in AD. Castellani et al. concluded that the choice of fixative is critical. They stated that the granular neuronal reactivity and the glial staining described by Takeda et al. are most likely artifacts of improper fixation.

Conclusive reinvestigation of glycation in A $\beta$ , NTF, and other cells and structures using consensus is needed to remove any doubts about the relationship of glycation to AD, and to evoke further research in this field. Studies must include age-matched controls.

#### 7.8. CSF studies

Shuvaev et al. studied changes in the level of an early glycation product, an Amadori product, in cerebrospinal fluid (CSF) in aging and late-onset AD [93]. The concentration of the Amadori product in CSF correlated with the CSF glucose concentration, but did not change with age ( $n=70$ ). In contrast, the level of CSF Amadori product was 1.7 times greater in AD patients ( $n=29$ ) than in a nondemented age-matched control group ( $n=20$ ;  $P<0.0005$ ). An increased accumulation of Amadori products was found in all major proteins of the CSF of AD patients, including albumin, apoE, and transthyretin. Glycation in albumin, normalized on protein quantity, was nearly 1.5 times greater in AD patients than in controls (total CSF glycation of the samples was 8.9 and 3.1 arbitrary units/ $\mu$ g of protein, respectively). This demonstrated that increased CSF glycation in AD is not due to a specific protein modification because all major CSF proteins of different origin are involved in the process (albumin from plasma, 90% of transthyretin synthesized by choroid plexus, and apoE derived from astrocytes). The site of high glycation in AD is not known. The CSF is likely to be at least one of the sites of excessive glycation, based on the hypothesis of the involvement of impaired CSF circulation

in AD. It is interesting to note that apoE4, the major genetic risk factor of late-onset AD, demonstrates a higher sensitivity to in vitro glycation than does normal apoE3, while glycated apoE maintains its high binding affinity to A $\beta$ -peptide.

Shuvaev et al. proposed that the increased early glycation of CSF proteins in AD patients might stimulate the formation and subsequent deposition of AGE as well as oxidative stress in the brain.

#### 7.9. Glycation product in serum

Riviere et al. quantified plasma protein glycation specifically derived from glucose in AD patients [75]. Protein glycation in plasma, evaluated by plasma furosine, was almost two times greater in subjects with AD than in controls, but still 50% less than in subjects with DM.

#### 7.10. In vitro studies

Recent in vitro studies also support the possibility of the involvement of glycation in neuronal cell death [40,52,107].

### 8. Glycation and oxidative stress

The following facts are known with regard to the relationship between glycation and oxidative stress (Fig. 4). (1) In the early stage of glycation, carbon atom (C) 1 of the sugar chain is reduced and C2 is oxidized. Since glycation is accompanied by oxidation, terminology such as 'glycooxidation' and 'glycooxidation products' (pentosidine, CML, etc.) is also used. In short, glycation is promoted under oxidative stress. (2) A large amount of reactive oxygen species is produced through glycation [29,34,59,78,97,119]. An Amadori product becomes a source of reactive oxygen species [97] when the Amadori product takes on an enaminol structure, a superoxide is generated via the Fenton reaction (mediated by transitional metal ions) and the Amadori product changes to glucosone.

Dopa and amino acid peroxides formed during glycooxidation also become sources of reactive oxygen species [18]. In experiments using cultured cells, reducing sugars such as ribose and fructose, which readily induce glycation, produce reactive oxygen species in pancreatic cells, resulting in their apoptosis [34]. Multiple lines of evidence suggest that oxidative stress plays an important role in the pathogenesis of AD [1,9,22,47,58,66,94,96,98,110], and oxidation may act in concert with glycation/glycooxidation [97,99]. These observations have led to the hypothesis that glycation-induced pathological conditions result from a series of oxidative stresses, increased modification of proteins via glycation, and further AGE-dependent oxidative stress [97].

## 9. Cytotoxicity of glycation on cultured cells

Experimental systems using cultured cells enable convenient examination of the relationship between glycation and oxidative stress. If glucose is used as the reducing sugar, a much longer incubation period might be needed to generate AGE, even in test tubes [23]. For this reason, we used a system of glycation acceleration based on the application of intermediate molecules, since cultured cells cannot be maintained over 2 weeks [40]. 3-DG and methylglyoxal (MG), intermediate products of glycation, are known to markedly accelerate glycation and AGE formation [12,51,90,117] (Fig. 4).

MG is one of a series of dicarbonyl compounds [12,51,68,102] that includes glucosone, deoxyglucosone, dehydroascorbate, and glyoxal, and is formed nonenzymatically by amine-catalyzed sugar fragmentation and by spontaneous decomposition of triose phosphate intermediates in glycolysis. It is also a product of the metabolism of acetol, an intermediate in the catabolism of both threonine and of the ketone body, acetone. MG reacts rapidly with the amino, guanidino, and thiol functional groups of proteins, leading to the denaturation and cross-linking of proteins. The physiological significance of protein modification by MG has been difficult to judge, since concentrations of MG at physiological levels could only recently be determined. However, Lo et al. have shown that MG binds and irreversibly modifies plasma proteins at physiological concentrations [51]. A novel MG-arginine adduct, argpyrimidine, has been identified and characterized. An immunohistochemical study showed the accumulation of argpyrimidine in some arterial walls of the rat brain after middle cerebral artery occlusion followed by reperfusion [71]. Glyoxalase-I catalyzes the conversion of MG to *S*-D-lactoylglutathione, which in turn is converted to D-lactate by glyoxalase-II [91].

3-DG [68,90,102,117], another highly reactive carbonyl compound, is formed after the dehydration and rearrangement of Amadori products. 3-DG reacts with free amino groups, leading to cross-linking in the late stage of the glycation reaction and resulting in the formation of AGE [16,62]. 3-DG-derived AGE are imidazolone, pyrraline, CML, and pentosidine, of which imidazolone is the most specific AGE for 3-DG. Elevated levels of 3-DG in plasma and erythrocytes promote the formation of AGE such as imidazolone, as demonstrated by immunohistochemistry and immunochemistry using an anti-imidazolone antibody.

Under diabetic conditions, the plasma concentrations of MG and 3-DG reach 5 and 1  $\mu\text{M}$ , respectively [72]. Niwa et al. reported the plasma concentration of 3-DG in diabetic patients with nephropathy as being 1235 ng/ml (7.6  $\mu\text{M}$ ), versus 314 ng/ml (1.9  $\mu\text{M}$ ) in healthy subjects [65]. These compounds are capable of inducing apoptotic cell death in the macrophage-derived cell line U937, at 10–300  $\mu\text{M}$  for MG and 10–1000  $\mu\text{M}$  for 3-DG [68]. In addition, PC12 cells are susceptible to the toxicity of MG

at 300  $\mu\text{M}$  and over, and to that of 3-DG at 10 mM and over [102]. Several reports imply a mechanism for the cytotoxicity of 3-DG and MG: (1) 3-DG and MG trigger cells to generate peroxides as judged by DCFH-DA and induce DNA ladder formation [68]; (2) in rat fibroblasts, DNA synthesis is suppressed [90]. This inhibitory effect is strengthened by glutathione depletion.

The following reports have been published regarding neurons. (1) In cultured dorsal root ganglion (DRG) cells, the generation of CML was detected after exposure to 3-DG and glyoxal. However, no evidence of neurotoxicity was observed [62]. (2) 3-DG induced toxicity in PC12 cells, which was suppressed by the overexpression of aldehyde reductase [102]. (3) The cytotoxic effects of AGE on BHK 21 hamster fibroblast cells and SH-SY5Y human neuroblastoma cells were examined [52]. AGE caused significant cell death in a dose-dependent manner. The cytotoxic effects were attenuated by antioxidants, aminoguanidine, and inhibitors of nitric oxide synthase (NOS). This suggests that not only ROS but also reactive nitrogen species contribute to AGE-mediated cytotoxicity (Fig. 4). (4) Neurite production and neuronal survival of cultured DRG neurons were significantly reduced on glycated collagen IV and laminin [54]. This report suggests that the involvement of components of basal lamina is one of the important factors for glycation toxicity.

## 10. Neurotoxicity of MG and 3-DG on cultured cortical neurons—synergism between glycation and oxidative stress

The effects of MG on neuronal survival were first examined morphologically. Incubation of cortical neurons with 100  $\mu\text{M}$  MG for 24 h resulted in a decrease in the number of cells as well as in the number of neurites and their length. Treatment with MG resulted in a concentration- and time-dependent decrease in the number of viable neurons when cell survival was assessed 24 h later. The  $\text{LC}_{50}$  of MG and 3-DG for neurotoxicity was 130 and 209  $\mu\text{M}$ , respectively [40].

Apoptosis was examined after 30 min by assessing chromatin condensation in cells stained with Hoechst 33258 for 30 min. Nuclear fragmentation began 30 min after exposure to MG, and continued to increase even after 3 h. DNA ladder formation was induced after incubation with 100  $\mu\text{M}$  MG for 3 and 24 h.

A higher level of peroxide was produced in cells treated with 50  $\mu\text{M}$  MG for 30 min than in those treated with vehicle, as detected using the fluorescence probe DCFH-DA. The neurotoxicity of MG and 3-DG was attenuated by *N*-acetylcysteine (NAC). NAC can raise intracellular GSH levels and thereby provide cells with the co-substrate required to eliminate hydroperoxides, resulting in protection from ROS. In addition, NAC also reacts with MG directly and reversibly to form the hemithioacetal adduct.

Co-treatment with aminoguanidine (AG) and 3-DG resulted in significant protection. The primary mechanism of AG is direct reaction with derivatives of Amadori compounds such as 3-DG, and prevention of subsequent AGE formation in susceptible proteins. In addition to its suppressive effect on glycation, AG has antioxidant properties [81] and also inhibits inducible NOS (iNOS) [100].

Niwa et al. reported that 3-DG and glyoxal accelerated the formation of CML in explant-cultured neurons in explant of dorsal root ganglia [62]. However, the authors did not fully explain the involvement of glycation in their system, since CML is produced by lipid peroxidation under conditions of oxidative stress, and the direct pathway from 3-DG to CML is a minor pathway. Even though glyoxal is a known precursor of CML, glyoxal itself is also formed during lipid peroxidation. In addition, the physiological significance of glyoxal has not been elucidated, even in diabetic complications.

Contrary to our data, neurotoxicity was not demonstrated in the study of Niwa et al. [62]. The concentration of 3-DG they used, up to 1 mM, was sufficient to bring about neurotoxicity in our study. Does this mean that CML is not sufficiently toxic to cause neuronal death? Several possibilities may explain the discrepancy, the most important being the difference in culture systems: our dissociated neurons in culture are efficiently exposed to 3-DG, whereas their explant neurons were surrounded by many non-neuronal cells that would likely have protective effects on the neurons.

## 11. Suppression of the cytotoxicity of glycation

Possible defense mechanisms against glycation in cells are outlined as follows. (1) Enzyme systems such as aldehyde reductase and glyoxalase detoxify 3-DG and MG, which are the building blocks of AGE. (2) The proteasome system and other protease–antiprotease systems degrade AGE-modified proteins. (3) Processing mechanisms through AGE receptors or AGE-binding proteins. (4) The macrophage system related to the disposal of AGE peptides, which act as seeds for protein aggregation. (5) Aminoguanidine and authentic agents that break cross-linking

Aldehyde reductase has been identified as a detoxication enzyme of 3-DG and MG, and has homology to aldose reductase. In PC12 cells overexpressing aldehyde reductase, 3-DG toxicity was suppressed as described above [102]. Aldehyde reductase, however, can be glycated and its enzymatic activity attenuated [103].

Chemicals such as AG are known to inhibit the generation of AGE. The primary mechanism of AG is direct reaction with derivatives of Amadori compounds such as 3DG, and prevention of subsequent AGE formation in susceptible proteins. In addition to its suppressive effect on glycation, AG has antioxidant properties [81] and also

inhibits inducible nitric oxide synthase (iNOS) [100]. OPB9195 is an inhibitor of AGE generation and is 10 times more potent than AG. Amadori product-degrading enzymes exist and are termed ‘Amadoriases’ [20,27,28]. *N*-Phenacylthiazolium bromide (PTB) and ALT711 are known as AGE cross-link breakers [111]. Vasan et al. reported preliminary findings suggesting that cross-link breakers can disaggregate amyloid deposits [111]. However, there is no evidence that drugs such as aminoguanidine can pass the blood–brain barrier. The importance of considering drug delivery should be emphasized in drug development. Amadorins were defined as inhibitors of the conversion of Amadori intermediates into AGE [38]. Pyridoxamine was identified as the first member of this class of Amadorins. AG has negligible Amadorin activity.

$\alpha$ -Lipoic acid is an antioxidant that inhibits the action of AGE [46]. Recently, it was clarified that acetaldehyde, a metabolite of ethanol, inhibits the generation of AGE [2]. Because some cells are damaged by activation of the receptor for AGE (RAGE), a trial to inhibit the synthesis of RAGE by ribozyme was conducted [109]. There is also a report that molecular chaperones such as  $\alpha$ -crystallin prevent glycation of enzymes [19,25].

## 12. Glycation in amyotrophic lateral sclerosis (ALS)

### 12.1. Familial ALS

#### 12.1.1. Familial ALS and SOD-1

Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative disorder that involves the motor neuron system. Approximately 10% of ALS cases (familial ALS, or FALS) are inherited; the other 90% are sporadic. Recent investigations have provided evidence that more than 50 mutations in the gene for SOD-1, the cytosolic copper/zinc-binding dimeric form of a protective metalloenzyme against injury by superoxide radicals, are linked to about one-fourth of the patients with autosomal dominant FALS. A discussion of FALS with SOD-1 mutations is complicated, because transgenic expression of different SOD-1 mutants in both mice and rats causes an ALS-like syndrome independently of whether SOD-1 catalytic activity is changed. These observations suggest that a novel gain-of-function effect of mutant SOD-1 may have a pathogenic role in FALS [33,85]. First, an increase in the peroxidase activity of mutant SOD-1 leading to hydroxyl radical production was assumed, while several authors report opposing findings [69]. Second, it is hypothesized that the formation of peroxynitrite via a copper-mediated mechanism accounts for FALS [5]. A recent report, however, strongly argued against the copper-mediated theory of motor neuron degeneration in SOD-1 mutant mice [101]. Finally, mutant SOD-1 has a tendency to form aggregates spontaneously [31,89].

### 12.1.2. Neuronal hyaline inclusions (NHIs)—A characteristic structure of FALS

Neuronal hyaline inclusions (NHIs), abnormal intracellular structures that appear in the soma and neurites of some of the surviving lower motor neurons and contain ubiquitin and phosphorylated neurofilament protein, are the characteristic markers of FALS with SOD-1 mutations. Shibata et al. [83] demonstrated the presence of intense SOD-1 immunoreactivity in the NHIs of FALS patients with a heterozygous Ala to Val substitution at codon 4 (Ala4Val) in the SOD-1 gene. Similar findings were observed in other ALS families with different mutations and a two base-pair deletion. Of added interest is a recent report that mutant SOD-1 expressed in cultured cells abnormally aggregates in the cytoplasm.

### 12.1.3. AGEs in NHIs

Shibata et al. investigated the immunohistochemical localization of CML, one of the major AGE structures, in spinal cords from three FALS patients with a heterozygous Ala to Val substitution at codon 4 in the SOD-1 gene [83]. In contrast to a weakly stained cytoplasm, the NHIs were intensely stained by a monoclonal antibody specific for CML. Immunoelectron microscopy revealed that the CML determinants were restricted to the granule-associated thick linear structures that primarily compose the NHIs. No focal collection of either CML or SOD-1 was found in neurons of the controls.

In the light of evidence that SOD-1 is a protein that is susceptible to the Maillard reaction, the finding of the coexistence of CML and SOD-1 in NHIs points to the possibility that CML-modified SOD-1 is deposited in NHIs. However, the target protein of CML modification in NHIs remains unclear because NHIs seen in patients with familial ALS have been shown to contain not only SOD-1 but also phosphorylated NFP and ubiquitin. At this point, it is of interest that, in general, cytoskeletal proteins such as NFP are subject to AGE modification because of their low rates of turnover. The decreased activity of SOD-1 by glycation is not necessarily considered to be the sole factor explaining the death of motor neurons.

### 12.1.4. AGE in astrocytic hyaline inclusions

Kato et al. [35,37] found SOD-1-positive inclusions in astrocytes (astrocytic hyaline inclusions, or AHIs) as well as in neurons (NHIs) in patients with FALS with SOD-1 mutations and in transgenic mice expressing human SOD-1 with the G85R mutation. AHIs were also immunoreactive for insoluble AGE such as CML, pyrraline, and pentosidine and, similar to NHIs, were ultrastructurally composed of granule-coated fibrils that exhibited immunoreactivity to CML and pyrraline.

### 12.2. Sporadic ALS

In cases of sporadic ALS as well as FALS, Chou et al.

[15] demonstrated the presence of glycation products in axonal spheroids of the spinal cord and ‘conglomerates’ in cortical motoneurons. Axonal spheroids and conglomerates are thought to be composed of neurofilaments. By means of specific antibodies, pyrraline, pentosidine, cypentodine/piperidine-enolone, and arginine-lysine imidazole were judged to be present in axonal spheroids and conglomerates. Pentosidine and pyrraline are frequently used as marker compounds of AGE formation in tissues.

In the report of Chou et al., no staining was detected in NHIs in ALS. Although this is a serious discrepancy between their data and the report by Shibata et al. [88], who found positive staining in NHIs, this could be explained by the specificity of the antibodies used: those of Chou et al. do not recognize CML and the AGE detected by Shibata et al. consisted of CML.

### 12.3. Familial vs. sporadic ALS/non-oxidative vs. oxidative glycation

CML is thought to be a major epitope for many of the currently available AGE antibodies. However, recent findings have indicated that a major source of CML may be derived from pathways other than glycation. Fu et al. clearly demonstrated that the major source of CML was lipid peroxidation, not glycation [18]. CML does not contain the reactive groups involved in cross-linking and is therefore less likely to be pathogenic. Thus, CML is considered to be a marker of oxidation rather than a product of glycation. Accordingly, the existence of CML is not decisive evidence of the involvement of glycation in the pathogenesis of ALS. It has been suggested that oxidative stress is closely linked to the formation of pentosidine [4]. Furthermore, pyrraline is thought not to function as a cross-linker [57]. To clarify the involvement of glycation in ALS and the interplay between glycation and oxidative stress, several studies were conducted, as discussed below.

Shibata et al. analyzed the immunohistochemical localization of CML as a lipid peroxidation or protein glycoxidation product, pentosidine as a protein glycoxidation product, and imidazolone and pyrraline as nonoxidative protein glycation products. They also analyzed 8-hydroxy-2'-deoxyguanosine (OHdG) as a nucleic acid oxidation product and acrolein-protein adduct and HNE-protein adduct as lipid peroxidation products. The analyses were conducted in the spinal cords of FALS patients with the SOD-1 mutation (A4V) in sporadic ALS patients, and in age-matched control individuals [86]. In the FALS cases, intense immunoreactivities for pyrraline and CML were confined to the characteristic NHIs, and imidazolone immunoreactivity was located in the cytoplasm of the residual motor neurons. No significant immunoreactivities for other examined products were detected in the FALS spinal cords. In the sporadic ALS cases, intense immunoreactivities for pentosidine, CML, and the HNE-protein

adduct were found in the cytoplasm of the degenerated motor neurons, and OHdG immunoreactivity was located in the cell nuclei of the residual neurons and glial cells. Shibata et al. also reported that immunoreactivities for markers of oxidation and/or glycoxidation (adducts of HNE, crotoaldehyde, CML and pentosidine) were localized in the gray matter neuropil of the spinal cord and in almost all of the motor neurons, reactive astrocytes, and microglia, but none of the immunoreactivities for non-oxidative glycation markers [87].

In addition, Kato et al. found that AHIs immunoreactive for CML, pyrraline, and pentosidine were negative for stress-response proteins (SRPs), HNE, acrolein, NOSs, and nitrotyrosine as representative markers of oxidative stress [35].

These results indicate that oxidative reactions are involved in the disease processes of sporadic ALS, while there is no evidence for increased oxidative damage except for CML deposition in the FALS spinal cord. Furthermore, it is likely that the accumulation of pyrraline and imidazolone supports the non-oxidative mechanism in the SOD-1-related degeneration of motor neurons.

However, their conclusion appears oversimplified, because glycation and oxidative stress are not mutually exclusive and many studies report the elevation of oxidative stress including increased nitration on proteins in FALS spinal cords. It is also highly possible that there are many 'non-oxidative glycation' markers other than pyrraline and imidazolone.

To answer the above questions, we first confirmed the initial stage of glycation in sporadic ALS spinal cords.

#### *12.4. An Amadori product, 1-hexitol-lysine, in sporadic ALS spinal cord*

We demonstrated the existence of an Amadori product, the initial reaction product of glycation, in the ALS spinal cord by the presence of an anti-1-hexitol-lysine (1-HL) antibody [61], and thus confirmed the involvement of glycation in this disease [39]. By observation of the anti-1-HL antibody, the presence of an Amadori compound was detected in axonal spheroids of the sporadic ALS spinal cord. A study of age-matched non-ALS spinal cords allowed us to conclude that 1-HL does not merely correlate with physiological aging, but is specific to ALS patients. Moreover, the observation of several brain regions other than the spinal cord demonstrated that the existence of 1-HL is prominent and specific in the spinal cord.

1-HL was also detected in the motoneurons of bulbospinal muscular atrophy (BSMA) spinal cords. Accordingly, glycation can be considered as a common pathway in motoneurons under pathological conditions. Although hyperglycemia is reported in some BSMA cases, and thus could enhance glycation in BSMA motoneurons, our cases of BSMA as well as of sporadic ALS showed no impairment of glucose tolerance.

We clearly showed the presence of an Amadori product, 1-hexitol-lysine, in the ALS and BSMA spinal cord. These findings suggest that subsequent advance of the glycation reaction results in the formation of AGE on proteins. The glycation reaction can act as a modulator of the death pathway of motoneurons in ALS and BSMA because glycation of essential components such as antioxidative enzymes and cytoskeletal proteins would cause dysfunction of these components.

#### *12.5. Late-stage glycation reaction in sporadic ALS*

Next, to investigate the existence of a late-stage glycation reaction, which proceeds to the production of AGE following the early reaction, in sporadic ALS spinal cords, the presence of CML and non-CML AGE was examined [41]. Prior to this research, we elicited antibodies that clearly distinguished non-CML AGE from CML [108]. Anti-non-CML antibody does not react with CML, pyrraline, pentosidine, or Amadori compounds, but possibly recognizes all other AGE. CML is thought to be a major epitope for many of the currently available AGE antibodies. However, recent findings have indicated that a major source of CML may be derived from pathways other than glycation [18]. Non-CML AGE, on the other hand, might play a more important role in pathogenesis by glycation. Examination of non-CML AGE is indispensable for clarification of the pathological significance of glycation.

In an immunohistochemical study, we noted intense positive staining with anti-CML in the cell bodies of the remaining atrophic motor neurons and in glia [41]. Anti-non-CML AGE antibody-positive materials were present in axonal spheroids and glial cells. Anti-CML and anti-non-CML AGE antibodies faintly stained vascular endothelial cells from both ALS and age-matched control subjects. With either antibody, corpora amylacea were negative in all cases. The existence of non-CML AGE was evident in the anterior horn of ALS spinal cords, demonstrating the existence of the late stage of the glycation reaction in ALS. The existence of CML was also confirmed, suggesting that augmented oxidative stress was also involved [41].

#### *12.6. AGE receptors in ALS*

Among the three types of AGE receptors (other than RAGE) reported in the human brain, AGE-R1 (oligosaccharyltransferase family) and AGE-R2 (substrate of protein kinase C) have been found in neurons, while AGE-R3 is restricted to glial cells. Chou et al. investigated the distributions of these receptors in conglomerates of cortical motor neurons in eight ALS brains (five sporadic ALS and three FALS) and three control brains with antibodies against AGE-R1 and AGE-R2 [13]. They found that AGE-

R1 immunoreactivity was co-localized with those of AGE, SOD-1, and neurofilaments.

### 12.7. AGE in an animal model of ALS

Large neurofilament-rich axonal spheroids in spinal motor neurons produced by chronic parenteral administration of  $\beta,\beta'$ -iminodipropionitrile (IDPN) were found to be immunopositive to AGE in the study of Chou et al. [114].

### 12.8. Cultured spinal neurons and glycation

We previously investigated the effects of two glycation-accelerating compounds, MG and 3-DG, on cultured spinal cord neurons [92]. Incubation of cortical and spinal neurons with MG or 3-DG for 24 h induced neuronal death in a dose-dependent manner. Spinal motor neurons were more vulnerable than spinal non-motor neurons and cortical neurons.

Przedborski et al. [73,74] reported reduced glutathione peroxidase (GSH-Px) activity in the brain region (precentral gyrus) affected in ALS. SOD and catalase activities were not changed, suggesting that specific defects of the GSH system are more important in ALS. Lucas et al. reported that the glutathione level was a crucial factor for the survival of spinal cord neurons in spinal cord injury caused by physical trauma and that glutathione augmentation might be an effective intervention strategy [53].

To test this possibility, we added glutathione-augmenting agents such as NAC and glutathione ethyl ester (GEE) 24 h before MG or 3-DG exposure in spinal cord cultures. All pretreatments showed significant protective effects. Increases in the survival of motor neurons were significantly greater than those of non-motor neurons. In contrast, the glutathione-depleting agents buthionine sulfoximine (BSO) and ethacrynic acid enhanced the toxicity of these compounds and caused much more prominent decreases in the survival of motor neurons compared to those of non-motor neurons. These lines of evidence suggest that free radical scavenging activity, particularly that of the glutathione system, might be limited or insufficient in spinal motor neurons.

This study showed the selective vulnerability of cultured spinal motor neurons to glycation-accelerating agents, which might reflect their acute toxic effects, and that this selectivity may be associated with the inefficiency of the glutathione system in spinal motor neurons. Since AGE accumulate in the spinal cords of ALS patients [14,84], further studies are needed to address the chronic phase of glycation toxicity resulting in the formation of AGE. These studies should employ experimental systems such as spinal cord slice cultures, which may provide longer survival for cultured tissue. It is also necessary to clarify the variations in the different types of defense systems against oxidative stress among the many types of neurons.

## 13. Conclusion

This review has outlined the glycation reaction including a discussion of some possible mechanisms of neurotoxicity. We give an overview of the role of glycation in the pathomechanism of AD. On the basis of the results of our in vitro studies using cultured cerebral cortical neurons, a synergism of glycation and oxidative stress is proposed. Defense mechanisms for glycation in cells are outlined in terms of neuroprotection. After reviewing glycation in ALS spinal cords, its neurotoxicity to cultured spinal motor neurons was elucidated. Since it is difficult to conclusively determine the pathological significance of glycation in neurodegenerative diseases, discussion will continue as to whether glycation is the primary and direct factor. However, whether primary or not, glycation might in some way be involved in neuronal death. If it is a modulating factor of the pathomechanism, interference with the process by which AGE formation occurs may provide new therapeutic opportunities to reduce the pathophysiological changes associated with neurodegeneration.

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