# Protein Glycation and Eye Diseases

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The major causes of blindness worldwide are diabetes and age related eye disorders. While the exact pathogenic mechanism for many of these disorders are not clearly known, there are evidences that protein glycation may play an important role in their etiology. Advanced glycation end products (AGEs) formed by Maillard reaction accumulate in the intracellular and/or extracellular environment of the ocular structure leading to crosslinking of various proteins, which may be involved in the development of various ocular diseases including cataract[1]. This review attempts to evaluate the link between AGEs, and various eye diseases such as diabetic retinopathy, cataract formation etc.

The non-enzymatic reaction between amino groups of proteins, lipids or nucleic acids and glucose or other reducing sugar results in the formation of a Schiff base that slowly rearranges to form the relatively stable Amadori product[2,3]. This reaction was first studied by Maillard in the early 1900 & is known as **Maillard reaction**[4]. This reaction was initially described in the context of food science where its products were found to impart changes in food texure, bioavailability, flavour & preservation. Maillard chemistry is now known to be very relevant invivo with important implications for health and disease. In the body the reaction between reducing sugars and / or carbonyls with amino group results in the formation of advanced glycation end products (AGEs) and these products then accumulate intracellularly and extracellularly on proteins, lipids and nucleic acids. Over the last 25 years enough evidence has accumulated to indicate that AGE modulation of these macromolecules represents a major factor in aging and in a spectrum of human diseases such as diabetic complications, neurodegenerations including Alzheimer's disease, atherosclerosis etc[5-9]. There is accumulating evidence that AGEs could play an important pathogenic role in eye diseases[1]. Ironically, Maillard's speculation made in the early nineties about the importance of this reaction in various diseases was too far ahead of time and received very little serious consideration.

The process of Amadori product formation is termed glycation and the protein bearing Amadori product is referred to as glycated protein distinguishing them from enzymatically glycosylated proteins. The Amadori product can undergo further oxidation or degradative reaction giving rise to additional protein bound compounds collectively termed AGEs[10].

The Amadori product can breakdown to form reactive alpha dicarbonyl compounds such as glyoxal, methyl glyoxal etc which cross link proteins[11].

Protein – protein crosslinking is thought to be responsible for a major share of the deleterious effect of AGEs in diabetes and ageing. Many of these AGEs have fluorescent properties which are used for their detection. Pentosidine, an advanced glycation end product, with fluorescent properties is one such AGE [13].

It is essential to maintain the structural integrity, optical clarity and adequate nutrition of the highly specialized cells of the eye for visual function. For eg., an opaque lens will prevent light from penetrating to the retina and thereby reduce visual acuity. Many of the differentiated cells of the mammalian eye have little or no regenerative capacity which makes them highly susceptible to the ageing process and systemic diseases that alter structural proteins. Ophthalmologist and various scientists have long recognized that eye is

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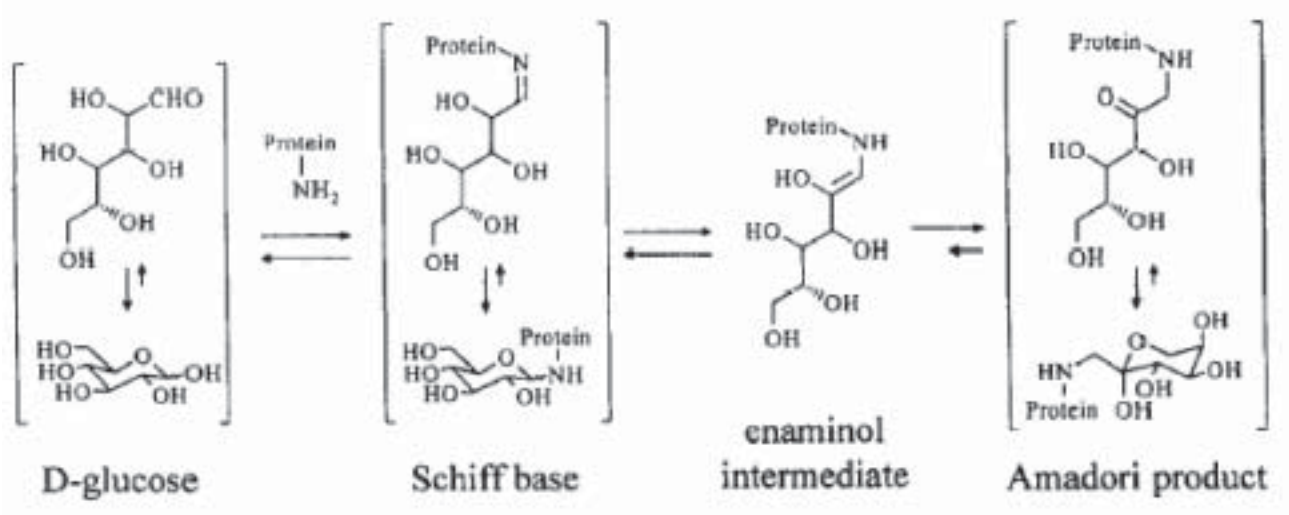


Fig. 1. Formation of glucose – protein Schiff base & the Amadori rearrangement.

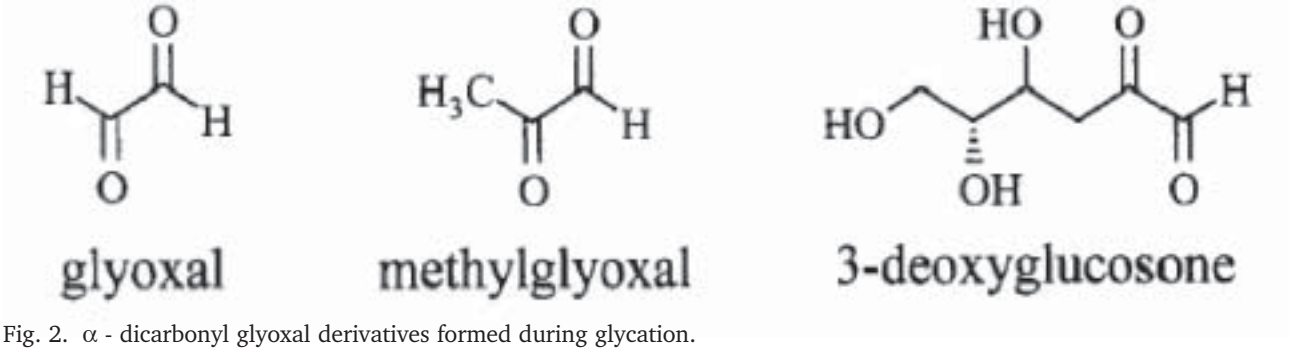


Fig. 2. α - dicarbonyl glyoxal derivatives formed during glycation.

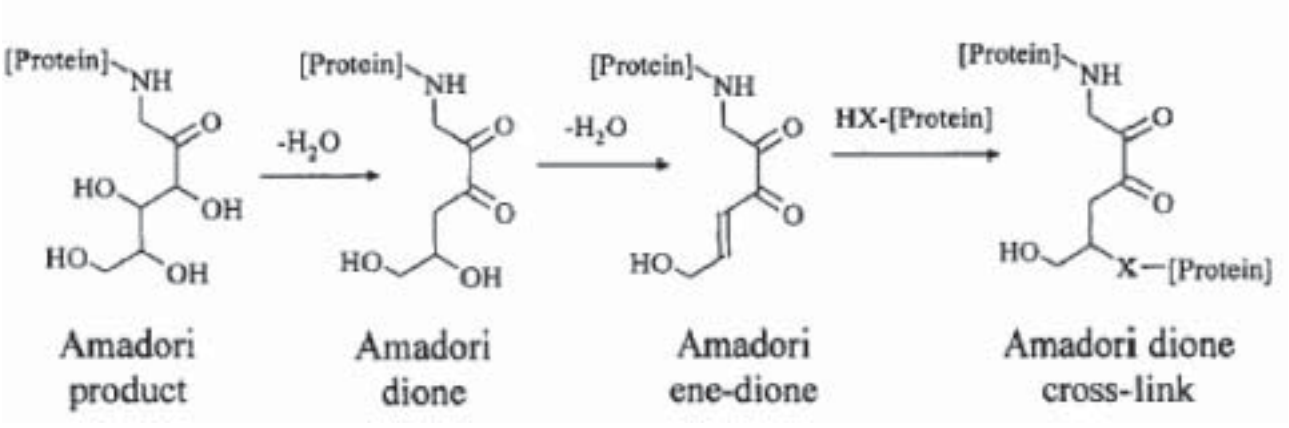


Fig. 3. Dehydration of the Amadori product to form Amadori dione and Amadori ene-dione and conjugate addition of a protein to form a protein-protein crosslink[12].

profoundly influenced by diabetes and age related dysfunction which together account for the leading cause of visual impairment world wide.

Lipid peroxidation can also form a class of Maillard products called advanced lipoxidation end products (ALEs)[14]. Indeed lipid peroxidation forming aldyhydes/carbonyl compounds which form Schiff's base with the amino group of proteins is important in the lipid rich, highly oxidative environment, such as in the retina and dyslipidemia may be an important factor in retinopathies.

Products of advanced glycation (AGEs) / lipoxidation (ALEs) are constantly formed under physiological conditions. Existence of complex receptor systems which bind these receptors have been suggested to instigate diabetic complication[15-17].

## AGEs in ocular tissues

### a. Cornea

As mentioned earlier, the role of AGEs in eye diseases is not well understood, but available data indicate that they have a role in age and diabetes related ocular disorders. The hyperglycemic state in diabetes promotes formation of AGEs which then produce alteration in structural proteins in the cornea leading to thickening of the corneal stroma & Descemet's / Bowman's basal laminae with morphological abnormalities in the epithelial and endothelial layers. [18, 19]

These alterations in the diabetic cornea are accompanied by decreased protein stability and increased immunoreactive AGEs[20, 21]. Bowman's membrane is highly glycated in diabetic patients[21,23]. Descemet's membrane is also very susceptible to AGEs [23].

AGEs accumulate also in the ageing cornea, as they do in the extracellular matrix proteins in other tissues[24, 25.]

### b. Lens

Cataract formation is by far the leading cause of visual impairment across the globe[26]. Ageing and diabetes are the major risk factors involved in cataract formation[27, 28].

The role of Maillard reaction in cataract formation has been extensively studied in both aged & diabetic lens where AGEs are significantly elevated[29 - 31]. Glycation generates age related alterations in lens fiber membrane integrity and tertiary structure of lens proteins, leading to aggregation and covalent crosslinking of lens crystallins. The action of dicarbonyls such as glyoxal and methylglyoxal is enhanced in diabetes and ageing leading to AGE cross links on a-crystallins with resultant loss of chaperone activity, increased ab-crystallin content and dense aggregate formation[32,33]. It is known that diabetic patients accumulate these products faster than age matched non-diabetic controls[34].

Smoking has been demonstrated to lead to high circulating levels of peptides in serum[35]. Tobacco smoking, which involves Maillard Chemistry, may be a rich source of reactive glycation products, capable of promoting AGE cross links formation invivo. In a study of cataractous lenses, a significantly higher level of immunoreactive AGEs has been demonstrated in patients with a history of smoking[36]. Smoking remains a clear risk factor for cataract formation[37] and tobacco related elevation in serum AGEs may act in concert with heavy metal deposition & oxidative stress to precipitate cataract formation. There is significant accumulation of copper in diabetic lens and copper catalysed Fenton's reaction results in generation of hydroxyl radicals with pathogenic significance in cataract formation[38].

### c. Vitreous

The vitreous gel that interfaces with retina is composed largely of complex net work of cross linked collagen and glycosaminoglycan (hyaluronic Acid)) [39].

Glycations can induce abnormal cross links between vitreous collagen fibrils leading to dissociation from hyaluronic acid and resultant destabilisation of gel structure[40]. AGEs accumulate in the vitreous of aged people and diabetic patients[41].

Even though nonglycational physiological and biochemical processes also contribute to vitreous degeneration, it seems likely that AGEs may play a significant role in diabetic and aging vitreous dysfunction.

AGEs are important in the development of age related macular degeneration (AMD). Evidence linking AGE accumulation in AMD can be surmised from the composition of several proteins such as vitronectin, TIMP – 3, Clusterin, apoliprotein E, amyloid[44-47] etc. Some of these proteins have been shown to be readily modified by AGEs and / or ALEs during aging.[48-51] AGE cross links accumulation is a feature of matrix dysfunction during diabetes. Bruch's membrane is known to thicken progressively in older patients and become less permeable[52-54].

AGEs causes apoptotic death in the cells of retinal pigment epithelium[55]. AGEs have been detected within the collagenous matrix of the lamina cribrosa and within the optic nerve head indicating their role in the pathogenesis of chronic open – angle glaucoma[56,57]. The lamina criborosa plays an important role in supporting the optic nerve axonal structure and AGEs mediated crosslinking of this matrix may reduce

flexibility and perhaps induce age – related axon damage characteristic of advanced glaucomatous disease[57].

**d. Diabetic Retinopathy**

Retinopathy is the most common microvascular complication of diabetes and remains an important cause of blindness[58]. With type 1 diabetes of 10 year duration, the prevalence of retinopathy is around 80% and increase to over 95% by 20 years[58]. Hyperglycemia is the underlying cause of the disease in both type 1 and type 2[59,60] diabetes In terms of Maillard products and diabetic retinopathy, clinical studies have demonstrated that the levels of AGEs in the serum[61], skin[62] or cornea[63] correlate with the onset or grade of diabetic retinopathy. AGEs are significantly increased in diabetic retinopathy patients.

AGEs are localized in retinal vessels and neuroglia of diabetic patients where they exert a range of deleterious effects on cell function[64-68]. Invivo and invitro studies suggest that elevated AGE level occurring in diabetes may be an important factor in retinopathy initiation and progression.

AGEs are known to cause significant upregulation of vascular endothelial growth factor (VEGF)[69-72] which is also a potent vasopermeability and angiogenic factor in the retinal microvasculature[73]. Extensive vasopermeability and angiogenesis are the pathophysiological hall marks of diabetic retinopathy.

## Therapeutic options in glycation mediated ocular diseases

Inhibition of Maillard reaction and prevention of AGE/ALE mediated cell toxicity have exciting possibilities.

The possible approaches are:-

(1) Inhibition of Amadori product formation

(2) Breaking of the preaccumulated AGEs

(3) Identifying substances with post Amadori product scavenging potential.

Amadori product formation is the crucial step in Maillard Chemistry in biological systems, because progression to crosslink requires chemical rearrangement to create reactive intermediates before the formation of irreversible AGEs. A **simple hydrazine compound, aminoguanidine (Pimagedine)**[74] has been shown to inhibit AGE mediated cross linking and to prevent a range of diabetic vascular complications in experimental animals including diabetic retinopathy[64-68].

**Aminoguanidine** has been evaluated in a multicentre clinical trial, where it showed positive signs towards slowing the progression of retinopathy[69]. However further extensive clinical trials are required.

Breaking the preaccumulated AGEs is an exciting approach. Two related compounds have been described to attack and break AGE cross links in experimental diabetes[70,71]. One is **ALT – 711** which has been reported to improve arterial compliance in aged patients with cardiovascular stiffness[72]. However the effect of ALT 711 on retinopathy is yet to be evaluated.

The third approach is to screen for compounds with post – Amadori product scavenging potential, since it is an important route for AEGs formation in vivo. **Pyridoxamine,** a derivative of pyridoxine has been described to be an effective and specific post - Amadori inhibitor with the ability to prevent renal dysfunction in diabetic rats. [73] It also reduced retinal AEG accumulation. [74]

To sum up, the Maillard reaction may play a pathogenic role in diabetic and age related dysfunction of the eye. The pathogenesis of such disorders is multifactorial and advanced glycation which may play a significant role, is not the only process leading to cell or tissue dysfunction. Important events in diabetic and aging such as free radical generation may also have important links to or are secondary consequences of Maillard Chemistry. Therefore any useful agent which may have therapeutic application in diabetic and age related ocular dysfunction should reduce glycation by decreasing hyperglycemia, inhibiting Amadori product formation and exerting antioxidant effect. *There has been one study on the curcuminoids from Curcuma longa on experimental cataractogenesis in rats with promising results*[75]. These are a number of medicinal plants which are reported to have hypoglycemic effect and antioxidant activity. These plants deserve a scientific study from the point of view of their antiglycating properties. There is immense need to unravel novel pharmacological intervention strategies to prevent / alleviate some of the sight threatening complications of diabetes and ageing.

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