Protein Glycation: A Firm Link to Cause Metabolic Disease and their Complications

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Abstract

Glycation of proteins starts with the formation of Schiff base, followed by intermolecular rearrangement and conversion into Amadori products. When large amounts of Amadori products are formed, they undergo cross linkage to form a heterogeneous group of protein-bound moieties, termed as Advanced Glycation End products (AGEs). The formation of AGEs is irreversible process, causing structural and functional changes in protein. This results in generation of free radicals which play an important role in pathophysiology of ageing and diabetes. The rates of these reactions are quite slow and proteins with large amounts of lysine residues undergo glycation with significant amounts of AGEs. The unwanted consequences of protein glycation may lead to several metabolic disorders like diabetes, arteriosclerosis, osteoporosis and Alzheimer’s disease etc. and their complications. This commentary reviews the glycation of proteins which have already been demonstrated by us and others.

Keywords: Advanced Glycation End Products (AGEs); Protein glycation; Pathophysiology; Diabetes; Arteriosclerosis; Osteoporosis; Alzheimer’s

Introduction

Glycation or a Maillard reaction is a post translational modification event which is the result of covalent bonding of a free amino group of proteins with a reducing sugar such as glucose and fructose which results in the formation of an early glycation product that undergoes rearrangement, dehydration and cyclization to form a more stable Amadori product (ketoamine) [1-4]. Both Schiff base and Amadori glycation products generate free radicals resulting in decline of antioxidant defence mechanisms which can damage cellular organelles and enzymes [5]. Under high glucose load (hyperglycaemic condition), the Amadori products undergo a non-enzymatic glycation reaction leading to the formation of a complex series of compounds known as the Advanced Glycation End products (AGEs) via intermediate compounds, such as 3-Deoxyglucosone (3DG), Glyoxal (GO) and Methylglyoxal MG [6]. Despite the fact that sugars are the main precursors of AGEs, these intermediary metabolites are also believed to participate in glycation reactions. Among these are 3-Deoxyglucosone (3-DG), known to be an important highly reactive dicarbonyl intermediate of the Maillard reaction; and Carboxymethyllysine (CML) and pentosidine as promoters of formation of AGEs. These intermediate compounds can also diffuse out of the cell and react with extracellular proteins. Excessive AGE accumulation results in significant cellular dysfunction by altering protein structure. Thus, 3DG, GO and MG are glycation intermediates and precursors of AGEs; and relevant targets for inhibitory compounds aimed to reduce the undesirable consequences of protein glycation both in vitro and in vivo. In addition to proteins, glycation affects a variety of other biomolecules containing free amino groups such as DNA and lipoproteins, thereby perturbing the structure and function of these biomolecules. The schematic representations of DNA glycation pathway along with protein and lipid macromolecule is given in Scheme 1.

Receptor for AGEs (RAGEs)

AGEs are the ligand for the cell surface receptor (RAGEs) which in turn mediates the downstream signaling pathway resulting in the formation of auxiliary free radicals. RAGEs are multi-ligand signal transduction receptors for AGEs of the immunoglobulin super family that mediate diverse cellular responses. AGEs may cause tissue injury directly via 1receptor-independent pathway or indirectly by binding to specific receptors for AGEs (RAGEs) on the surface of various cells. The binding of AGEs on the RAGEs induces activation of nuclear factor-κB(NF-κB), resulting in increased expressions of cytokines, growth factors and adhesion molecules involved in the pathogenesis of various diseases [7]. Therefore, these glycation processes has to be stopped using various inhibition studies. Few amongst them could be the use of medicinal plants having anti-oxidant properties.

Protein glycation overview

The dominant factor in protein glycation is the half-life of individual proteins; greater the half-life, larger the glycation [8,9] The role of glycation proved to be significantly responsible in many proteins for their structural deformity and their functions. The target proteins are like; Immunoglobulin G (IgG), HSA, collagen which causes unwanted consequences. IgG and albumin having half-lives of around 21 and 20 days, respectively, exhibit utmost in vivo glycation. However, at high glucose concentration, the extent of glycation is mainly determined by intrinsic glycability of protein.

Glycation of Immunoglobulin G (IgG) has been implicated in autoimmune diseases such as Rheumatoid Arthritis (RA) [10]. This may interfere with the normal function of IgG and may contribute to initiation of arthritic complications. AGEs damaged IgG may be used as a biomarker for early detection of RA and the associated secondary complications. Glycation of IgG is of special interest due to its influence on the functionality of immunoglobulin and overall immune competence, especially with regard to their ability to bind antigens and induce the complement cascade. Glycation of immunoglobulin has been shown to cause major structural perturbations resulting in their functional disability [10].

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Another serum protein is human serum albumin (HSA) which is highly susceptible to glycation. HSA is the most abundant protein that comprises 60% of human plasma protein. It has been used as a model protein for protein folding and ligand binding studies over many decades due to its long life period and high concentration in plasma. HSA is highly sensitive to glycation because HSA bears approximately 58 Lys residues, making it a favourable target for the glycation process [11]. The level of glycated albumin might also be of value as an indicator of the degree of hyperglycaemia in diabetics [12].

In recent years lipo-protein glycation has been studied in great detail. The non-enzymatic binding of reducing sugar to lipoprotein can increase the atherogenic potential of Low-Density Lipoprotein (LDL) [13]. The cause for atherogenicity is due to the glycation reaction of Apo-lipoprotein B (apo-B), a surface protein of LDL macromolecule. LDL glycation is significantly increased in diabetic patients compared to normal subjects, even in the presence of good glycemic control. Diabetic patients undergo in vivo glycation of apo-B due to elevated blood sugar levels. It has been shown that glycation alters the biological activity of LDL resulting in reduced rate of degradation by fibroblasts, which increases cholesteryl ester deposition in human aortic cells compared with normal LDL [13]. This gave rise to the hypothesis that lipoprotein glycation contributes to the accelerated atherosclerosis in diabetic patients. Development of diabetic complications that enhances glycation causes structural alterations that in turn lead to functional abnormalities of LDL [14-16]. Recently, the glycation of histone proteins have received potential significance [17]. The bio-accessibility of the sugar molecules like, glucose and other carbonyl compounds in and around the nucleus of the cell makes histones a soft target.

Among the extra cellular protein, collagen is the most important one to undergo glycation reaction. Collagens are important proteins for the skin, as they are essential for structure and function of the extracellular matrix in the dermis. Thinner and wrinkled skin, the typical signs of normal ageing, is the consequence of reduced collagen. Protein glycation especially collagen, due to its long half-life, contributes to skin ageing as it deteriorates the existing collagen by cross linking [18].

Why study Glycation?

The study of biological macromolecule glycation represents one of the most promising areas of research today. Apart from diabetic microvascular disease, AGEs have been implicated in a wide and seemingly disparate range of pathologies such as connective tissue, particularly in rheumatoid arthritis (RA) and neurological conditions such as Alzheimer’s disease (AD) and End-Stage Renal Disease (ESRD) [19]. In vitro work has shown AGEs to be a part of complex interactions within oxidative stress and vascular damage, particularly in atherosclerosis [20] and in the accelerated vascular damage that occurs in diabetes [21].

Increased glycation and build-up of tissue AGEs have been implicated in diabetic complications because they can alter enzymatic activity, decrease ligand binding, modify protein half-life and alter immunogenicity. Some recent studies have reported the presence of auto-antibodies against DNA and protein-AGEs in the serum of diseased individuals [15,16,22]. AGEs are capable of forming AGE-immune complexes in diabetic patients that may play a role in atherosclerosis. Glycation-derived free radicals can cause protein fragmentation and oxidation of nucleic acids and lipids. AGEs could also form on phospholipids and induce lipid peroxidation by a direct
reaction between glucose and amino groups on phospholipids such as phosphatidyl ethanolamine and phosphatidyl serine residues.

The effect of glycation on lysine rich proteins and their involvement in aging and age-related diseases has been reviewed scrupulously [23]. The evidences support antigenicity of the glycated lysine residues in vivo with observation of auto-antibodies against the glycated proteins in diabetes and RA patients. This could be due to protection of the modified proteins from proteolytic breakdown and its recognition as a foreign molecule by the immune system. A greater understanding of the regulation of glycated lysine products, especially Amadori products, in aging may play an important role in preventing the risk of age-related diseases. Thus, auto-antibodies to glycated proteins pose a marker for future age related diseases in presently healthy individuals [24]. Our research team has probed the presence of auto-antibodies against the carcinoigen/free radical and glycated DNA molecule as a probable bio-marker for the detection of early onset of the diseases like diabetes, arthritis and cancer as well [25-29].

The study of glycation becomes important because AGEs progressively accumulate on tissues and organs developing chronic complications of diabetes, retinopathy, neuropathy and progressive atherosclerosis.

**Diabetic secondary complications**

**Retinopathy:** The lens of eye is a thin homogenous, retractive epithelial basement membrane in the form of a capsule and is made up of glycoprotein and collagen produced by the epithelial cells. The lens capsule collagen contains higher quantities of hydroxlysine than the interstitial collagen. The amino group of amino-terminus, C-group of lysine residue and C-hydroxyamino group of hydroxyl lysine residue are potential sites of glycation in proteins [30]. Hydroxylysine residue also present in the lens epithelial basement membrane [31] and their prolonged exposure to the aqueous humour glucose can cause a substantial non-enzymatic glycation. The glycation rates in a crystalline increases as a result of aging and diabetes but remains almost constant in β and γ crystalline. This non-enzymatic glycation of proteins causes opacification of the lens, leading to cataract formation.

**Neuropathy:** Diabetic neuropathy is another diabetic secondary complication associated with the non-enzymatic glycation reaction under hyperglycemic condition leading to the AGEs formation. The AGEs have shown to accumulate in myelin and tubulin of peripheral nerves. Non enzymatic glycation of such neural protein is thought to impair axonal transport, which may induce diabetic neuropathy [32]. In the peripheral nerve, persistent hyperglycaemia leads to metabolic and vascular disorders responsible for nerve fibre abnormalities.

**Nephropathy:** Diabetic nephropathy cannot be escaped when one talk of slow and sweet reaction, the glycation. In this case, glucose-dependent metabolic pathways and vasoactive hormones may directly influence tubular and interstitial cells, leading to renal dysfunction caused by non-glomerular mechanism [33]. High intracellular glucose level lead to the enhanced formation of AGEs in particular CML modified protein. Therefore, the level of CML and pentosidine AGEs are measured in nephropathic patients undergoing peritoneal or haemodialysis in order to correlate it clinically. This is the reason why the research in glycation biology becomes more important and targeted in case of metabolic disorders.

**Inhibitors or antiglycating agents:** Protein glycation and formation of advanced glycation end products (AGEs) have been reported to play an important role in the pathogenesis of various diseases like diabetes, rheumatoid arthritis, osteoporosis and aging [34]. Thus, the intermediates and precursors of AGEs are most pertinent targets for compounds aimed to reduce the detrimental consequences of protein glycation both in vitro and in vivo.

Aminoguanidine (AG) is an archetype therapeutic agent for the prevention of formation of advanced glycation end products. It reacts rapidly with alpha, beta-dicarbonyl compounds such as MG, GO and 3-DG to prevent the formation of advanced glycation end products (AGEs). The adducts formed are substituted 3-amino-1,2,4-triazine derivatives [35]. The notion that accumulation of AGEs is a risk factor for disease succession has been substantiated by inhibition of vascular complications in experimental diabetes by AG. However, the clinical trials on AG was stopped because of safety concerns, as it was found to be nephrotoxic at a concentration at which it inhibited the formation of AGEs.

The Pyridoxamine (PM), a vitamin B6 metabolite, has proven to be a potent inhibitor of the formation of AGEs in in vitro and animal experiments [36]. This effect of PM is most probably due to blockage of the oxidative degradation of the glucose derived Amadori intermediates or due to quenching of the dicarbonyl compounds [37]. It inhibits the progression of renal disease and decreases hyperlipidemia and apparent redox imbalances in type 1 diabetic rats [38].

Naloxone, made up of thebaine that is obtained from plant *Papaver somniferous* [39], inhibits the formation of AGEs and is purported to have therapeutic potentials in patients with diabetes and age-related diseases [40]. Similarly, there are various other medicinal plants which might have anti-glycation potential.

Benfotiamine, a pro drug of thiamine monophosphophate, has AGE-lowering properties without decreasing early glycation adducts [41]. Benfotiamine as well as thiamine have been reported to reduce diabetic nephropathy and retinopathy in experimental animal models [42,43]. Benfotiamine has been shown to rectify multiple pathways of biochemical dysfunction, and its major intrusion in AGE formation in vivo, is by preventing dicarbonyl formation [44]. Administration of benfotiamine to type 2 diabetic patients, on a high AGE content diet, reduced circulating AGE levels and markers of oxidative stress [45].

**Conclusions and Future Prospective**

Increased non-enzymatic protein glycation, formation of AGEs and their accumulation in tissue and serum have an important role in the pathogenesis of diabetes complications. Long- lived extracellular proteins have highlighted the importance of intracellular glycation. The chemical nature of AGEs, their synthesis in vivo and their precise role in the pathogenesis of diabetes complication is under investigation. The diabetic complication can be reduced by reducing glycation synthesis cross-link formation and tissue accumulation of AGEs or by blocking AGEs receptors. Recently our group has focussed in the inhibition of AGEs using potential drugs like metformin and pyridoxamine [46]. Numerous natural and synthetic compounds are being investigated for their possible therapeutic potential having anti-oxidant potential [47-51]. We also hypothesized that medicinal plants having anti-oxidant potential might also prove to be have good anti-glycation capability [52]. The compounds like, aminoguanidine prevent formation of AGEs and have proved promising in the prevention of diabetic complications in animal models. However, they could not be developed into an effective marketable drug, due to safety considerations. Better understanding of the molecular mechanism, responsible for diabetic complications is necessary for development of the inhibitors of AGEs.

Therefore, need of the hour is to synthesize potential drugs which are peptide/protein specific and should have least or no toxicity to the
nearby cells or tissues [53]. Our research group has also done recent developments in the inhibition of bone cancer cells using glycation assisted synthesized gold nanoparticles [54]. Therefore a journey from macro to nano is also utmost important if one wants to halt the menace of this sweet and slow non-enzymatic glycation reaction [55].

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