Amadori Glycated Proteins: Role in Production of Autoantibodies in Diabetes Mellitus and Effect of Inhibitors on Non-Enzymatic Glycation

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ABSTRACT: Nonenzymatic glycation of macromolecules, especially proteins leading to their oxidation is increased in diabetes mellitus due to hyperglycemia and play an important role in associated complications of the disease. The glycation primarily occurs at intrachain lysine residues of proteins and results in the formation of an early stage stable product as Amadori-lysine which undergo further irreversible chemical reactions to form advanced glycation endproducts. This review deals with the role of Amadori modified proteins in pathogenesis of diabetes. We aim to explain immunogenicity of Amadori-glycated proteins, which might be involve in production of serum autoantibodies in the diabetic patients, and effect of inhibitors on the glycation process.

Key words: Amadori products, Antibodies, diabetes complications, lysine rich proteins

Hyperglycemia induced non-enzymatic glycation is initiated by a nucleophilic addition reaction between a free amino group of a protein and a carbonyl group from a reducing sugar to form a freely reversible Schiff base. This reaction occurs over a period of hours and once formed, the labile Schiff base rearranges to a more stable Amadori product (a ketoamine or fructosamine) (1, 2), which occurs as cyclic forms (pyranose or furanose) in equilibrium for added stability (Figure 1) (3, 4). Glucose-derived Amadori product reacts with itself or primary amines, such as the ε-amino-lysine and undergoes further reactions to form advanced glycation end products (AGEs) (5, 6). Nε-carboxymethyllysine (CML) and pentosidine, and glucosepane and crossline, are the AGEs formed through oxidative and non-oxidative pathways, respectively. The covalent bond of sugar-protein complex is formed through a series of chemical reactions known as Maillard reactions (7).

Non-enzymatic glycation of proteins

Glycation of proteins take place at ε-amino groups of lysine or hydroxyllysine residues as well as at α-amino groups of amino terminal residues (8). Specific lysine residues in hemoglobin, human serum albumin and α-crystallins have been identified as preferential sites of glycation. Other lysine-rich proteins, IgG and IgM, were found to be glycated in diabetes patients (9, 10). Glycation also takes place on arginine residues (11) and that of histidine, tryptophan and cysteine residues (12). Amadori modification of hemoglobin was done by the use of glyceraldehydes (13) and synthetic peptides were synthesized with lysine residues as the specific site for Amadori modification (14).
Figure 1. A scheme showing cyclic form of Amadori products during non-enzymatic glycation of proteins [Adapted from Bioorg Med Chem, 16, Adrover M, Vilanova B, Frau J, Munoz F, Donoso F, The pyridoxamine action on Amadori compounds: A reexamination of its scavenging capacity and chelating effect, 5557-5569, Copyright (2008), with permission from Elsevier (37)].
Table 1. Amadori products of proteins in diabetes and diabetic complications

<table>
<thead>
<tr>
<th>Amadori products</th>
<th>Diabetes mellitus</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histones</td>
<td>Diabetes</td>
<td>15</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>Type 1 diabetes with retinopathy &amp; nephropathy</td>
<td>16, 17</td>
</tr>
<tr>
<td>Collagen</td>
<td>Diabetes with retinopathy</td>
<td>18</td>
</tr>
<tr>
<td>IgG, IgA, IgM</td>
<td>Type 1 &amp; 2 diabetes with nephropathy</td>
<td>22</td>
</tr>
<tr>
<td>12 plasma proteins (e.g. albumin, fibrin, transferrin)</td>
<td>Type 2 diabetes</td>
<td>23</td>
</tr>
<tr>
<td>Albumin, Lipoproteins</td>
<td>Diabetes with atherosclerosis</td>
<td>24</td>
</tr>
<tr>
<td>Phosphatidyl-ethanolamine</td>
<td>Diabetes</td>
<td>28</td>
</tr>
</tbody>
</table>

Amadori modified proteins in diabetes and associated complications

Amadori-glycated lysine-rich proteins represent a potential marker for hyperglycemia in diabetes mellitus. HbA1c, an Amadori product of hemoglobin and glucose, is used to monitor long term blood glucose control in diabetic patients. Amadori product of a variety of these proteins was found to be associated with diabetes and its complications. Examples for these types of proteins (intracellular and extracellular) are presented in Table 1. Amadori modified histones were identified in liver cells of diabetes patients (15) whereas Amadori albumin was associated with early nephropathy and with retinopathy in type 1 diabetes patients (16, 17). Increased level of Amadori-glycated collagen was found in type 1 diabetic patient with or without retinopathy and it was also independently associated with retinopathy (18). It is reported that Amadori products of glycated serum proteins contribute to diabetic nephropathy (19, 20) and elevated concentrations of Amadori albumin in animals were linked with diabetic retinopathy (21). Fructosamine levels of purified immunoglobulins (IgG, IgA, IgM) were higher in both type 1 and 2 diabetes patients with nephropathy as compared to those without any complications (22). A number of Amadori-modified plasma proteins such as immunoglobulin (Ig) heavy-chain constant regions and Ig light chains were found in type 2 diabetes patients with the help of Amadori-antibody (1-deoxyfructosyl lysine) (23). Amadori glycated albumin and lipoproteins were associated with increased atherosclerosis in diabetes (24). A detailed study has been done for Amadori product in relation with diabetes complications (25). We have used glucose modified poly-L-lysine to determine the presence of Amadori adducts of lysine rich proteins in diabetes mellitus (26, 27). Recently, higher levels of Amadori products of phosphatidylethanolamine, a lipid, were found in blood of diabetic rats (28). In most of the above studies the diagnostic methods for detection of Amadori-specific antibodies were based on solid phase enzyme immunoassay like ELISA, Western Blot and Gel-Retardation.

Immunogenicity of Amadori-glycated proteins

Majority of the glycated proteins in plasma exist as Amadori products rather than in the more labile Schiff base form (29), and only a small part of the Amadori products undergo subsequent rearrangements to AGEs. Anti-hexitol lysine IgG, a polyclonal antibody raised in rabbits, was used to detect Amadori modified proteins in tissues of normal and diabetic rats (30). Amadori products of HSA have been reported to be highly immunogenic in experimental animals and anti-Amadori albumin antibodies recognize cyclic form of the Amadori product (16). We have shown the presence of antibodies against glycated lysine residues in sera of diabetic patients (both type 1 & 2) with the use of Amadori-rich glycated poly-L-lysine (Figure 2) (26). Most of the glycation related serum autoantibodies, discovered in diabetes patients with or without diabetic complications, were directed towards AGE-modified proteins such as IgG, BSA and HSA (9, 31, 32). However, there is a need for investigations on the presence of antibodies against Amadori-glycated proteins in diabetes patients with or without diabetic complications. These antibodies would be of great help in early diagnosis of the disease.
Figure 2. Reactivity of autoantibodies to native and modified poly-L-lysine (PLL) in diabetes patients. Reactivity of serum antibodies from diabetes mellitus (DM) patients to native PLL (□), glycated PLL (■) and NaBH4 reduced glycated PLL (▓). Serum samples from normal human subjects (NHS) served as control. The microtitre ELISA plates were coated with the respective antigens (10 μg/ml). *p < 0.001 vs. native poly-L-lysine (PLL) [Adapted from Human Immunol, 70, Ansari NA, Moinuddin, Alam K, Ali A, Preferential recognition of Amadori-rich lysine residues by serum antibodies in diabetes mellitus: Role of protein glycation in the disease process, 417-424, Copyright (2009), with permission from Elsevier (26)].

Inhibitors of glycation

High level of fructosamine was obtained in glucose modified collagen by the use of inhibitors for oxidation and cross-linking (33). Amadori adducts formation in glucose modified HSA were inhibited by penicillamine (34). Intensive insulin treatment decreases Amadori modification of albumin, fibrin, transferring, Ig heavy chain constant region and Ig light chain (23). The importance of lysine residues in the glycation process has been supported by the investigations showing lysine mediated inhibition of glycation (35, 36). While the use of aminoguanidine is discontinued in clinical studies, role of pyridoxamine as a potent post-Amadori inhibitor is still not clear (37, 38). Other glycation inhibitors like nucleophilic amines/compounds, pyridoxamine, thiazolium salts are not yet studied extensively for their side effects. These inhibitors are mainly preventing formation of AGEs but they have no effect on formation of Amadori products. Reports have suggested that soluble receptor for AGE (sRAGE) or RAGE antagonist could be a future therapeutic target for inhibiting glycation (39).

More recently, effectiveness of current pharmacological inhibitors (Figure 3) for the glycated products has been studied in detail (40). It showed inhibitory role of a new molecule, GLY-230 (2-[3-chlorophenylamino] phenylacetic acid), on Amadori modification of albumin and ALT-711 (3-phenacyl-4,5-dimethylthiazolium chloride) as an AGE-cross-link breaker. Several plant products like caffeic and chlorogenic acids in herbal mate tea (41) and S-allyl cysteine in aged garlic extract (42) have shown promising effects towards inhibiting AGE formation. Rutin, a flavonoid in fruits and vegetables, and its metabolites inhibited early glycation product as well as AGEs (43-45). A significant inhibition of glycated hemoglobin and glycated albumin was obtained by phytochemicals of Cranberry (46). The newly developed inhibitors for the protein glycation were gold and silver nanoparticles (47-50). Gold nanoparticles effectively inhibited glycation of α-crystallins, collagen and HSA.
while silver nanoparticles inhibited AGE-induced retinal complications. The above studies indicate that Amadori modification of lysine residues causes structural perturbation in the proteins and are involved in overall immunogenicity of a glycated protein. This might lead to the recognition of Amadori-glycated proteins by serum autoantibodies in diabetes patients. Inhibition of the formation of these Amadori products by using natural sources and nanoparticles, which have minimum side effects, might be an early step in prevention of the disease complications.

![Figure 3. Inhibitors for the products of non-enzymatic glycation of proteins.](https://example.com/figure3)

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**References**


