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Minireview

The role of glycation in aging and diabetes mellitus

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Abstract

One of the hypotheses trying to explain the process of aging is the idea of glycation of proteins. This reaction, also called the Maillard or browning reaction, may explain age-related symptoms such as cataract, atherosclerosis and modification of collagen-containing tissues. Diabetics, which posses elevated blood sugar levels, show signs of accelerated aging exposing similar complications. The Maillard reaction, which occurs on a large scale in vivo, may play a key role in the initiation of these symptoms.

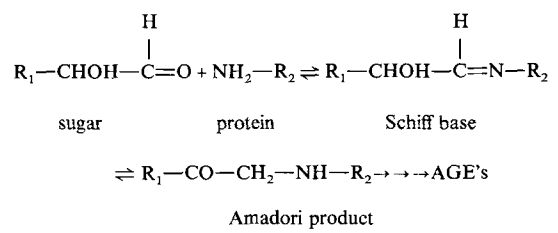
Introduction

Glycation is a reaction between sugars and proteins which takes place without the aid of enzymes. The reaction was first described by Louis Maillard [1], who observed the browning of protein samples upon heating with sugars. For many years, the 'Maillard reaction' seemed only important for food chemistry. Not until 1975 discoveries were made which revealed the relevance of glycation in vivo. Glycation seems to occur in every living organism. It is especially important in individuals with elevated blood-sugar levels such as patients with diabetes mellitus and galactosemia. Proteins with a long half-life are more intensely subjected to this modification process.

Recent research has made clear that glycation in vivo has serious consequences for the biological functions of proteins. Thus, glycation seems to play a role in both, aging and the pathological complications of patients with diabetes mellitus.

Chemistry

The first step in the glycation process is the reaction between the aldehyde group of a reducing sugar and the free amino group of a protein (this can be the terminal amino group or the ε-amino-group of lysine). The result is a still labile Schiff base, which undergoes a rearrangement to form the more stable Amadori product.



The Schiff base and Amadori product are called the early glycation products. The sugar involved determines the rate of glycation; some sugars are more reactive than others [2–5]. Glucose, the

most abundant sugar in living tissue, has a relatively low reactivity. Phosphorylated sugars and carbohydrates with a short carbon chain are very reactive. Recent experiments [5] show a remarkably high reactivity of the compounds dihydroxyacetone and glyceraldehyde and their phosphates which, together with glucose-6-phosphate and fructose-6-phosphate, occur on key positions in the anaerobic glycolysis. In vitro experiments with lens proteins have shown a possible role for ascorbic acid in the modification of proteins [6,7]. Not ascorbic acid itself, but its oxidation products dehydroascorbate and diketogulonic acid seem to be responsible for this type of Maillard reaction [8].

The temperature, nature of the incubation medium, accessibility of lysine residues, half-life of the protein and, especially, the sugar concentration are important in the levels of protein modification. The steps following the ketoamine formation are not yet elucidated. A large number of reactions follow, resulting in complex carbohydrate-protein complexes. These structures can possess chromophoric and fluorophoric properties and are able to crosslink protein chains. These compounds are called advanced glycation endproducts or AGE's (stressing the importance of these products in the aging process). The crosslinking properties of AGE's is one of the most important consequences of glycation.

Some time ago, the structure of a glycation endproduct was proposed being a furoyl-furanylimidazole compound [9]. Profound investigations have shown that this product was generated during the isolation procedure and is not actually present in vivo [10–12]. Only recently the structure of an AGE compound was elucidated [13,14]. This crosslinking group, which the authors called pentosidine, is the condensation product of an arginine residue, a lysine residue and a pentose. It occurred in a large number of tissues, in long living proteins as well as in proteins with a short half-life. The compound was present in high levels in tissues with high metabolic activities, reflecting the high levels of pentose turn-over. In the human lens, with its low metabolism, only minor amounts were present. Hayase et al. detected immunologically an acid-labile hydroxymethyl pyrrole aldehyde group (which they called pyrraline) on glycated proteins [15]. Levels of protein pyrraline immunoreactivity were clearly elevated in diabetic subjects. The pyrraline compound has the highly reactive 3-deoxyglucosone as intermediate (see below). Probably large numbers of glycation endproducts are present in vivo. Though much effort has been undertaken in a search of fluorescent products, crosslinking and fluorescent groups are not necessarily linked properties of AGE's [16,17]

Physiological consequences

The glycation of a protein has dramatical results. Early glycation products will change the isoelectric point of the protein. This change is even more profound when the sugar involved contains a phosphate group. As a result of these changes in charge, the molecule can undergo conformational changes. In vitro, this has been shown for the eye lens proteins, the crystallins [18]. Especially incubation with glucose-6-phosphate resulted in a clear change in the conformation of these proteins as revealed by circulair dichroism. Advanced glycation products will cause crosslinking of protein chains. In the case of long-lived proteins, this can lead to serious complications. In aging individuals, a significant decline in glucose tolerance is observed. Age seems to be an independent determinant of glucose tolerance: the decline is a primary aging effect and not secondary to other age-associated characteristics [19]. The sugar concentrations play a crucial role in the levels of protein modification. This indicates the importance of glycation in the etiology of patients with diabetes mellitus and galactosemia. The accelerated rate of glycation in these patients probably gives rise to a wide range of complications.

Glycation of plasma proteins

The first observation of in vivo glycation concerned the modification of hemoglobin. In 1962,

Huisman & Dozy [20] showed that the HbA1 fraction (containing three minor components of human hemoglobin) was increased in diabetic patients. Later studies by Fluckinger & Winterhalter [21] demonstrated that HbA1c could be easily formed in vitro by incubating hemoglobin with glucose. It became clear that the level of HbA1c was closely linked with the rate of glycemia of the patient [22–24]. Since the early eighties, the measurement of glycated hemoglobin has been used clinically as an indicator of glycemia in diabetic patients. Recently, the measurement of glycated albumin seems to become more common practice [25,26]. Since albumin has a shorter biological half-life compared with hemoglobin (20 and 120 days, respectively), this assay is a short-time indicator of glycemia. Glycation of albumin in vivo occurs at multiple sites, Lys-525 being the principal site of modification [27,28]. Other plasma proteins, such as immunoglobulins [29], erythrocyte membrane proteins [30], and low-density lipoproteins [31] were found to be glycated in vivo. Glycation of LDL had a profound effect on the plasma clearance; modifications in the range of 3 to 6% of lysine residues inhibited the clearance up to 20% [32]. Glycated low-density lipoproteins and high-density lipoproteins are possibly involved in the development of atherosclerosis, a common feature in patients with diabetes mellitus [33,34].

Glycation of long-lived proteins

Crystallins

The level of modification of a protein depends on the time it is present in the body. Proteins with a long biological half-life are, therefore, longer subjected to this process. Lens crystallins posses a unique property: they have no turn over at all. Once synthesized, they remain in the lens during the total life-span of the individual. As aging proceeds, the crystallins become more and more modified by different posttranslational modifications [35,36]. In patients with diabetes as well as in aging individuals, cataract is a rather common feature. During cataract formation, the crystallins aggregate to form high-molecular-weight material, which has severe consequences for the transparency of the lens. As glycation causes crosslinking and aggregation of proteins, it might possibly contribute to the development of cataract. In animals, there seems to be a correlation between the levels of protein glycation and the amounts of high-molecular-weight material [37,38]. The glycated proteins are enriched in the high-molecular-weight fraction. Human lenses from diabetics show a clear increase in glycation products when compared with healthy individuals [39–41]. Glucose is not the only sugar involved in the glycation of crystallins; 10–20% of the in vivo bound hexose in human crystallins was connected via C-2, indicating that the proteins had reacted with fructose [4]. In vitro experiments support the hypothesis of conformational changes and formation of high-molecular-weight material due to glycation [17,42,43].

An alternative theory on cataract formation is the idea of the polyol pathway, introduced by Kinoshita [44,45]. When in the lenses of diabetic individuals glucose levels are elevated, this sugar is converted into sorbitol by the enzyme aldose reductase. As sorbitol is unable to cross the lens cell membranes, the osmotic stress in these cells is increased dramatically. This could contribute to the damaging of lens fiber cells and the development of cataract. Many experiments show that the effect of diabetes, except for the increase in the lens of glucose and glucose-6-phosphate, is a flux of glucose into the polyol pathway initiated by the enzyme aldose reductase. Consequently, the flow of glucose through the pentose phosphate pathway is enhanced, restricting the glycolytic route in the diabetic lens [46]. The importance of the polyolpathway was underlined by experiments which show the prevention of non-tryptophan fluorescence and high-molecular-weight protein formation by the aldose reductase inhibitor sorbinil [47]. Interestingly in this respect, is recent work which revealed the presence of fructose-3-phosphate in the lenses of diabetic rats [48]. This sugar phosphate, which is not present in normal lenses, is a glycating agent and an enzyme inac-

tivator. Fructose-3-phosphate is labile and produces upon hydrolysis a highly reactive dicarbonyl compound, 3-deoxyglucosone.

Collagens

Another group of long-lived body proteins are the collagens. Though some investigators doubt if the low levels of glycation products in vivo could result in relevant physical changes in collagens [50], other workers point out that glycation might take place at biologically important sites of the molecule, such as the collagenase site or glycoprotein interacting sites [51]. The glycation of the glomerular basement membrane is particularly well investigated [52–54]. The consequences are, in the case of these basement membranes, more severe compared with fibrous collagen; flexibility of the membrane and permeability of the capillaries are clearly affected [51]. The binding of fibronectin and heparan sulphate to basement membrane collagens is crucial in their functioning in filtration. Interestingly, investigators have reported a strong reduction in affinity of these proteins to glycated type IV basement membrane collagen [55]. Investigations by Tsilibary and co-workers [56] suggest that glycation of the main non-collagenous domain NC1 (located at the carboxyl end of type IV collagen molecules) may interfere with normal assembly of type IV collagen in diabetes mellitus and is possibly related to abnormal functions of basement membranes in this pathological condition. Many of the observations were done following in vitro glycation; the relevance of these processes in the living organism still have to be elucidated. As with other body proteins, the levels of early glycation products are elevated for skin collagens in patients with diabetes mellitus; glycation products were also present in normal individuals, in an age-related way [57–60].

Not only the early glycation products are elevated, but also the fluorescent groups increased dramatically with age and in diabetes. The only identified structure of an advanced glycation product, pentosidine, was first isolated from human collagens [13]. It is clear that in vitro glycation of collagens has consequences for its stability, solubility and resistance to enzymes [61–64].

Not only internal glycation of collagen occurs, there is also evidence for combinations of collagen with non-collagen-protein. Collagens in arterial walls have shown to accumulate AGE's and trap normally short-lived proteins [62,65,66]. As a result, the arterial walls are thickened. Thus, glycation may explain the increased appearance of atherosclerosis in both, aging individuals and patients with diabetes.

Osteocalcin

Also bone protein, osteocalcin, was found to be glycated in vivo in an age-related way [67,68]. It was established that the site of glycation was the amino-terminal tyrosine [68]. Glycated osteocalcin in the bones of aging individuals possibly plays a role in the pathogenesis of senile osteoporosis and in osteopenia, seen in diabetes mellitus.

Myelin

One of the characteristics of diabetic secondary complications is peripheral neuropathy, showing segmental demyelination. Myelin has shown to be highly glycated in diabetes mellitus [69,70]. Increased permeability of diabetic endoneurial capillaries to plasma proteins, resulting in trapping of these proteins [66], may result in peripheral nerve damage. The permeation of glycated albumin into the endoneurium of the sciatic nerve of rats was significantly greater compared with normal albumin [71]. Glycated albumin enhances its permeation into the nerve, which may be relevant in the development of diabetic neuropathy. The possible role of macrophages (possessing receptors for AGE's) in this process is discussed below.

Nucleic acids

In recent years, pilot experiments have been done to investigate the possibility of glycation of the

amino groups of DNA bases. Upon in vitro incubation of DNA with reducing sugars, it could easily been shown that chromophores and fluorophores arise with similar spectral properties when compared with protein-AGE's [72,73]. The reactions proceeded faster with single stranded DNA than with double stranded DNA. This modification of DNA has biological consequences; the transfection potential of f1 phage decreased clearly following in vitro glycation with glucose or glucose-6-phosphate [72]. Further evidence for biological relevance of DNA glycation came from experiments which showed the mutagenic effect of glucose-6-phosphate on plasmid DNA [74]. Some investigators suggest the presence of a precursor (composed of lysine and a reducing sugar) before the actual reaction with DNA takes place [75]. Others propose a role for radicals (via auto-oxidation of reducing sugars) in the modification of DNA [73,76].

Glycation and oxidative processes

Some investigators argue against the relevance of the Maillard reaction in vivo by pointing out that the extent of protein glycation is small in absolute terms: after 120 days (the average lifespan of an erythrocyte) only 1% of the total amino groups on hemoglobin is glycated. In diabetic subjects, this figure increases to about 2.5% [77]. Alternatively, Wolff and co-workers developed a theory involving free radicals combined with elevated levels of sugars in diabetics. In vitro, small amounts of transition metal seemed to be required for sugar-induced non-tryptophan fluorescence [78,79]. The authors suggest an auto-oxidation of glucose, generating peroxides, free radicals and highly reactive dicarbonyl compounds.

These dicarbonyl compounds (such as 3-deoxyglucosone, which is formed following the Amadori conversion [80]) are held at least in part responsible for the modifications of proteins in the presence of glucose [81].

In vitro experiments with 3-deoxyglucosone produced fluorescent products, accelerated polymerization and caused a decrease in enzymatic digestibility of the proteins [82]. In rats, administered 3-deoxyglucosone was not biologically utilized, but rapidly excreted in the urine [83]. Part of the compound was converted into 3-deoxyfructose [83]. Studies of the model compound N(α)-formyl-N(ε)fructoselysine (fFL) revealed an oxidative cleavage into N-carboxymethyllysine and erythronic acid. This reaction seems also to take place in vivo and occurs by a free radical mechanism [84]. Carboxymethyllysine was detected in human lenses and tissue collagens [16,84]. Carboxymethyllysine and N-(carboxymethyl)-hydroxylysine (the product of oxidation of glycated hydroxylysine) were found in human skin collagen in an age-dependent way [85]. This oxidative cleavage of the Amadori-product may limit the accumulation of advanced glycation products in vivo.

Inhibition of the Maillard reaction

A number of investigators tried to find suitable non-toxic glycation inhibitors. Aminoguanidine, a nucleophilic hydrazine compound has frequently been studied in its capability to prevent glycation-induced crosslinking. When administered to rats, aminoguanidine was able to prevent diabetes-induced formation of fluorescent advanced glycation products and inhibited crosslinking of arterial wall connective tissue protein [62]. Recent work shows that aminoguanidine does not work on the protein, but decreases the concentration of the active aldehyde form of the sugars [86]. Aspirin (acetylsalicylic acid) has an effect on the formation of early and advanced glycation products in crystallins [43,87–89]. When aspirin was fed to rats, formation of lens opacification was considerably delayed, glycation levels decreased as well as formation of high-molecular-weight aggregates, while blood glucose levels remained the same [89]. Other agents which have shown to be effective in inhibition of the glycation of proteins are reduced glutathion [43], Ibuprofen (2-[4-isobutyl-phenyl]-propionic acid) [49,88,90], Bendazac (1-benzyl-[^{1}H]indazol-3-yl oxyacetic acid) [91] and pyridoxal-5-phosphate [92].

There is evidence that the body itself is able to trace, capture and degrade proteins containing advanced glycation products. Cerami and coworkers observed that macrophages were able to bind and degrade AGE-containing proteins (AGE-BSA) via a specific cell-surface binding protein [93]. This receptor was thought to recognize the putative furoyl-furanyl-imidazolium compound (FFI), identified by the same investigators. The binding protein recognized the carbonyl group, furan rings and an imidazolium structure and had a molecular weight of 90 kD [94]. Since later experiments revealed that the imidazolium compound does not occur in vivo, the involved AGE-structure must resemble the structure of FFI. When formaldehyde-modified albumin (f-Alb) is injected in rats, a rapid plasma clearance is seen [95]. The f-Alb receptor involved was seen by the authors as a general scavenger receptor for aldehyde-modified proteins. The cellular binding of AGE-BSA in cell cultures mentioned above, was indeed inhibited by addition of f-Alb [96,97]. f-Alb had also a profound in vivo effect on the plasma clearance of AGE-BSA in rats. Remarkably, FFI and its BSA-conjugates did not influence binding or plasma clearance of the AGE-BSA, which makes the involvement of FFI in the receptor-mediated uptake of advanced glycation products unlikely [96].

The removal and uptake of AGE-proteins seems to be associated with the production and secretion of TNF (tumor necrosis factor or cachectin) and IL-1 (interleukin-1) [98]. The authors suggest that crosslinked senescent proteins are removed by cytokine-dependent proteases after recognition by macrophages which initiate secretion of cytokines like TNF and IL-1. Recent data report an upregulation of the macrophage/monocyte receptor for glycated proteins by cachectin/TNF [99]. AGE's induced human monocyte chemotaxis and the secrection of platelet-derived growth factor [100]. These data support the hypothesis that AGE's can act as signals for the turnover of senescent proteins via a specific receptor system.

Though glycation has a profound effect on proteins in vitro and the reaction occurs in every living organism, it is not yet clear to which extent it is responsible for the complications seen in diabetics and aging individuals. Glycation occurs on a large scale in vivo with a most apparent effect on long-lived molecules. Possibly, glycation is just one factor in a series of modifications. Remarkably, food restriction in rats shows retarded age-related processes, increase of mean and maximal lifespan and lower levels of glycated hemoglobin [101,102]. These results, together with the fact of faster development of age-related processes in diabetics, strongly support the idea of a role for reducing sugars in the aging process.

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