

Review

Protection against glycation and similar post-translational modifications of proteins

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Abstract

Glycation and other non-enzymic post-translational modifications of proteins have been implicated in the complications of diabetes and other conditions. In recent years there has been extensive progress in the search for ways to prevent the modifications and prevent the consequences of the modifications. These areas are covered in this review together with newer ideas on possibilities of reversing the chemical modifications. © 2006 Elsevier B.V. All rights reserved.

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1. Introduction

Proteins exist in an environment surrounded by reactive small molecules and it is inevitable that these molecules will react with the proteins even without the benefit of enzymic catalysis. The small molecules include metabolites, especially sugars; and xenobiotics, including toxins and pharmaceuticals [1]. The reaction with sugars, glycation, has been investigated most carefully because it plays a major role in diabetic complications and other diseases, but the consequences of other post-translational modifications can be broadly similar, although investigation of the means of prevention is less advanced.

2. Glycation

Glycation is the non-specific reaction of sugars with proteins, and proceeds without the need for an enzyme. It happens wherever protein is in contact with sugar. Any protein will in time react with any reducing sugar, but some sugars are more reactive than others and some protein groups are more reactive. Glucose is the least reactive sugar in these uncontrolled pathways and this may have led to its central role in metabolism [2].

Glycation increases with age as do sugar concentrations, impairing protein function. In diabetes, sugar concentrations, the extent of glycation and the degree of damage increase in parallel. Glycation may be the main cause of diabetic complications.

2.1. The glycation reactions

The glycation reaction starts as the simple formation of a Schiff base between a sugar in its open chain form and a protein amino group and proceeds to a complex set of reactions to form coloured, fluorescent and crosslinking species called advanced glycation end products (AGEs). The early glycation reactions are shown in Fig. 1. The first step leads to a Schiff base (glycosylamine), which then undergoes the Amadori rearrangement to form a ketoamine. After the Amadori product the reactions become more varied and complicated leading to the AGEs. The structures of some AGEs are shown in Fig. 2. Formation of some AGEs such as carboxymethyllysine requires an oxidative step after the glycation. The emphasis of research in recent years has been on AGEs [4] but early glycation products can have a damaging effect and may be more important in damaging non-structural proteins such as enzymes [5], and serum proteins [6].

Experimental studies have utilised a wide variety of proteins and many sugars from glucose, the least reactive, and other hexoses, to sugar phosphates, ribose, methylglyoxal, glyoxal,

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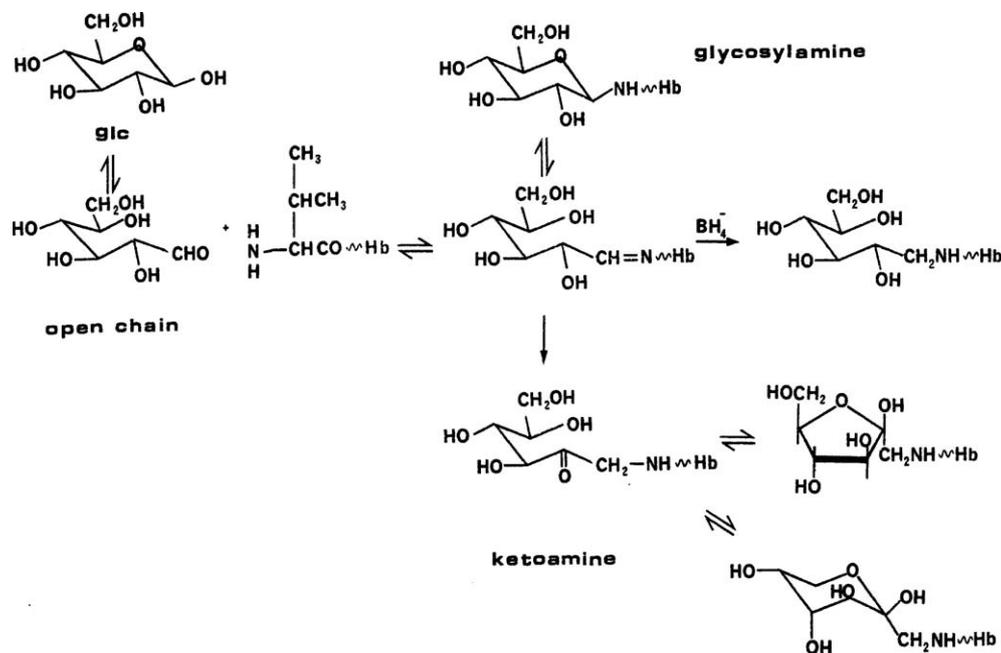


Fig. 1. Early glycation reactions: the formation of the Schiff base and Amadori compound. Taken from [1].

ascorbate and its oxidation product dehydroascorbate and other sugars [5]. The reactivity of individual sugars depends largely on the proportion that exists in the open-chain form. Some major AGEs may be derived from lipid [7].

2.2. The effects of glycation

Investigation of glycation-induced damage to proteins associated with ageing and complications of diabetes was mostly on long-lived structural proteins, because they are exposed to the sugars for a longer time than enzymes and other non-structural proteins in most tissues. The extent of glycation was approximately doubled in diabetes for a variety of proteins including collagen, myelin basic protein, lens crystallins, lens capsule, LDL, as well as haemoglobin [1]. Similar increases were found in ageing. Glycation caused conformational change to the proteins [8] and, under harsher conditions, yellowing, aggregation and crosslinking associated with the increased presence of AGEs [9,10]. Although the emphasis has been on long-lived proteins even proteins that turn over rapidly like insulin and enzymes are damaged by glycation [5,11]. In many instances proteins damaged by the inevitable glycation are simply turned over and cause no harm.

The extent of glycation, and especially of AGE formation, increased with the severity of diabetic complications [12–15].

Furthermore glycation increases in the lens with age and cataract and in other diseases such as Alzheimer's disease [5,16].

2.3. Other post-translational modifications of proteins

Although glycation has been more widely studied than any other non-enzymic post-translational modification of proteins to occur in vivo there are many other modifications of importance. Reactions with aldehydes and ketones are chemically similar to

glycation and have similar effects [1]. Binding of corticosteroids falls into this category.

3. Protection against glycation

Sequelae of diabetes include heart, kidney, nerve, lens and retina damage and over the years evidence has accumulated that diabetes is also a risk factor for other age-related diseases such as Alzheimer's disease and a variety of cancers [17]. Much of the damage may be caused by glycation. As understanding of the damaging effects of glycation has grown there has been an increasing interest in protecting against it either by prevention or by dealing with the consequences of glycation. Pharmacological prevention of diabetic complications [18,19] and prevention of glycation of insulin [11] were reviewed recently.

3.1. Prevention of glycation

The obvious way to prevent excessive glycation in diabetes is to control blood sugar levels more tightly and this has been shown to decrease the progression of the sequelae of diabetes such as retinopathy [20,21]. Curiously Stratton et al. showed that just as many subjects had significantly improved retinopathy as had worse retinopathy after 6 years. Unfortunately they did not analyse for the factors associated with the improvement. These may well have included glucose and glycated haemoglobin levels but may have included consumption of aspirin and other agents to be discussed below. In rats severe dietary restriction decreases glycation, but this result could not be repeated in primates and the idea is not popular in man [18].

There are several types of agent to prevent glycation: some may compete for the amino groups on the protein, some may bind to the protein to prevent access to the amino groups, some

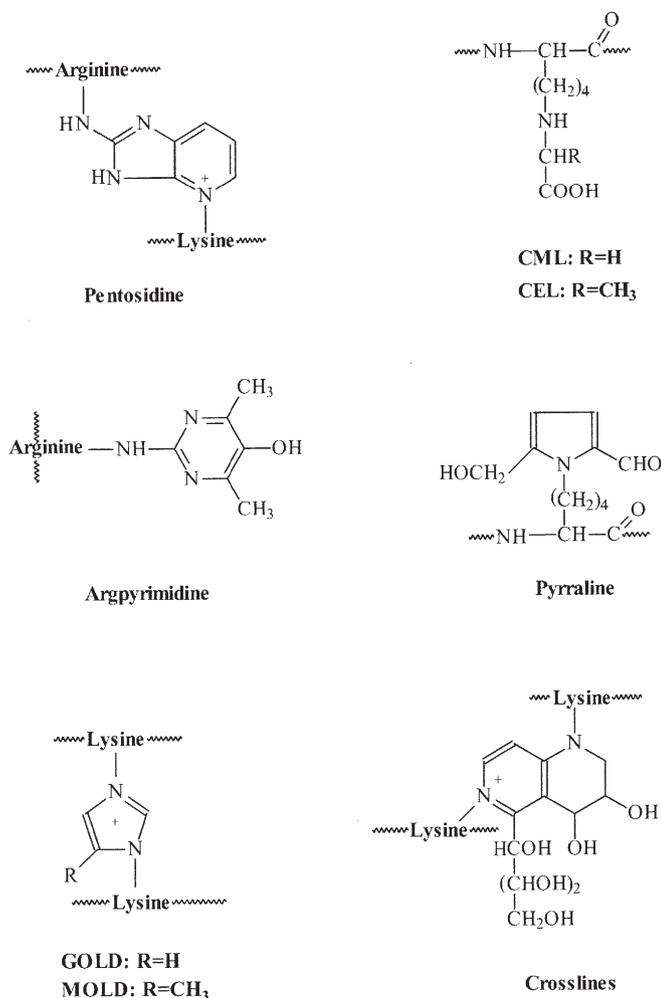


Fig. 2. Structures of some AGEs: pentosidine, argpyrimidine, carboxymethyl-lysine (CML), carboxylethyllysine (CEL), pyrroline, glyoxal-lysine dimer (GOLD), Methylglyoxal-lysine dimer (MOLD) and crosslines. Taken from Jakus and Rietbrock [3] with kind permission of the authors and publishers.

may simply mop up the open chain form of the glycating sugars, and some may bind to a glycation intermediate to prevent progress to the AGEs (Fig. 3; Table 1).

Aspirin was probably the first molecule shown to prevent glycation. When present in incubations of lens proteins aspirin prevents glycation by galactose [22], glucose [23], glucosamine [24] and other sugars. It appeared that the protection by aspirin against glycation and similar post-translational modifications was by acetylation of the reactive amino groups on the proteins [25–27]. All crystallin groups were acetylated and the modification was stable. More recently a single acetylated lysine was identified in a human crystallin [28]. Thus aspirin would inhibit at 'a' in Fig. 3.

Then it was found that other anti-inflammatory drugs, ibuprofen and diclofenac, also protected against glycation by glucosamine, galactose and fructose [9,24,27–30]. As ibuprofen and diclofenac have no acetyl group to transfer, acetylation cannot be the only explanation of the protection. Aspirin-like drugs also protect some enzymes against glycation-induced inactivation, e.g., malate dehydrogenase [31], delta-levulinic dehydratase [32], catalase and fumarase [33].

Aspirin, ibuprofen and paracetamol (acetaminophen) were shown to protect against cataract in diabetic rats [22,34]. There is evidence that these well known drugs also afford some protection against human cataract [35–40]. These protective effects may be a result of preventing glycation.

Analogues of diclofenac without the ability to inhibit cyclooxygenase have been synthesized and shown to inhibit glycation, as a result of which they also prevent the development of diabetic nephropathy in the *db/db* mouse [41].

In addition to the benefits for cataract there is increasing evidence for beneficial effects of aspirin-like drugs in ischaemic heart disease, stroke, Alzheimer's disease, colorectal cancer and prostate cancer, adding strength to the view that these diseases may have overlapping aetiologies and that it may be possible to find common therapies [42]. It may be that glycation is part of the common aetiology.

Compounds that react with the sugars and remove them from reaction with protein have been proposed as anti-glycation agents. These include amino acids [43], polyamines [44] and peptides such as carnosine [45,46]. These would be type b inhibitors in Fig. 3. Carnosine and other peptides could protect aspartate aminotransferase, Cu,Zn-superoxide dismutase and an esterase against glycation-induced inactivation [47–49]. In fact carnosine can also react with protein carbonyls produced by glycation and thus decreases crosslinking (Fig. 3d) [50]. There is also evidence that carnosine can disaggregate glycated protein [51]. Preliminary evidence from a small trial of carnosine in Russia indicated that it might be a useful therapy for cataract [52]. The tripeptide glutathione can also prevent glycation in vitro [22,24] probably by forming Amadori adducts with the sugars as have been identified with glucose and ribose [53,54] (Table 1).

Of course a treatment that mops up the open chain form of sugars will have the potential for harm in that this form is used for vital metabolic reactions. On the other hand most compounds proposed in this group are natural compounds. Careful attention must be given to dosage but the balance of hazards will only be discovered when and if these compounds reach clinical trials. It could be argued that acetylation of proteins by aspirin might be hazardous but there is long experience of aspirin as a drug and its side effects are well known.

As the Amadori product (Fig. 1) is quantitatively the most important glycation product in lens [18] it is reasonable to try the agents mentioned above to prevent its formation.

The next point of intervention is to prevent the later reactions that lead to AGEs, and the first compound studied in that way was aminoguanidine, which was supposed to react with the Amadori product and block further reactions [54–56] making it a type e inhibitor (Fig. 3). However it became clear that aminoguanidine also prevents the original glycation (type b) [57,58]. Now it is thought of more as a way to eliminate methyl glyoxal and other dicarbonyls and as a metal chelator, intervening at d and e in Fig. 3 [10,18,59]. Although charged, aminoguanidine is able to penetrate into cells [58]. Aminoguanidine forms stable 3-amino-1, 2,3-triazine adducts with dicarbonyl compounds (Fig. 4) [60].

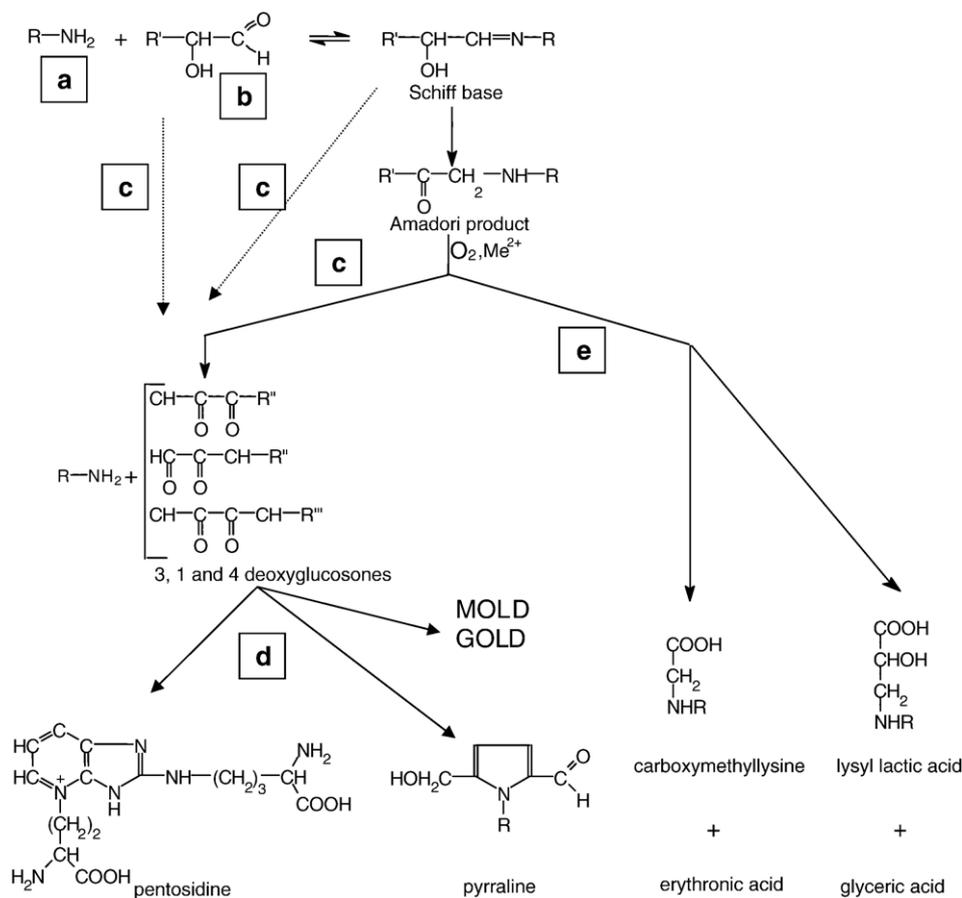


Fig. 3. Possible sites of intervention by anti-glycation agents. Taken from Ganea [11] by kind permission of the author and publisher.

Aminoguanidine prevented the diabetes-related increase in protein kinase C which may play a role of its own in diabetic complications [61]. Whether aminoguanidine prevented early or late glycation it was effective in decreasing cataract development, maintaining nerve conduction velocity and preventing albuminuria in diabetic rats [14,61,62]. It also decreased carboxymethyl-lysine accumulation in heart and aorta of aged rats [63]. Its early successes supported the view that glycation is largely responsible for diabetic complications, but side effects observed during clinical trials have blocked further progress [59].

Metformin, which is widely used to lower blood glucose in type II diabetes, has a biguanide structure (Table 1) related to aminoguanidine and decreases glycation in vitro, including AGE formation, probably by eliminating reactive carbonyl intermediates [64–66]. High doses of metformin appeared to decrease plasma methyl glyoxal levels in diabetic subjects without affecting glycated haemoglobin [67]. The metformin-methylglyoxal adduct was identified as a triazepinone [68]. If metformin is a type d inhibitor (Fig. 3) this accounts for it having no effect on glycated haemoglobin, which represents early glycation products. Buformin has a similar structure (Table 1) and a similar effect on AGE formation in vitro [66].

Other compounds able to prevent early and late changes include penicillamine [55,69], pyridoxamine [18,70,71], an aminoguanidine-pyridoxal adduct [72], pioglitazone, pentoxi-

lylline [64] and thiamine pyrophosphate [70] (Table 1). It was suggested that pyridoxamine, like aminoguanidine, may act largely by mopping up methylglyoxal [73]. It minimized the raised carboxymethyl-lysine and protected against pathological changes in diabetic rat retina [74]. A newer compound, OPB-9195 or [(+/-)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide] (Table 1), prevented the formation of carboxymethyl-lysine and pentosidine in vitro when serum albumin was incubated with glucose, ribose or ascorbate [75]. It also prevented formation of the dicarbonyls glyoxal, methylglyoxal and 3-deoxyglucosone. In diabetic rats it kept AGE levels closer to normal, normalized nerve conduction velocity and protected sciatic nerve NaK-ATPase [76]. Another hydrazine compound, ALT-946 or *N*-(2-acetamidoethyl)hydrazinecarboximidamide hydrochloride, inhibited AGE crosslinking in vitro and in vivo, protecting against albuminuria and AGE deposition in kidney [77].

In human red cells the NaK-ATPase can be inactivated by glycation [78] and lipoic acid can protect against this loss of function and also protect Ca-ATPase, and lower glycated haemoglobin levels [79].

Olmesartan and other angiotensin II type I receptor antagonists, as well as captopril and other ACE inhibitors, decrease formation of AGEs more effectively than aminoguanidine and as widely used hypotensive drugs they have great potential as therapy against diabetic complications [80].

Table 1
Agents that protect against glycation

Prevention of glycation
Aspirin (acetylsalicylic acid)
Ibuprofen
Diclofenac
Amino acids
Polyamines
Carnosine
Glutathione (gamma-glutamyl-cysteinyl-glycine)
Aminoguanidine
Metformin and buformin (biguanides)
Penicillamine
Pyridoxamine
Aminoguanidine-pyridoxal adduct
Pioglitazone
Pentoxifylline
Thiamine pyrophosphate
OPB-9195 ((+/-)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide)
ALT-946 (N-(2-acetamidoethyl)hydrazinecarboximidamide hydrochloride),
Lipoic acid
Olmesartan and other angiotensin II type I receptor antagonists
Captopril and other ACE inhibitors
Pyruvate
Dilazep
Anserine
Cysteine,
Cysteamine
Sulphite
3-mercaptopropionate

Pyruvate protects against glycation of α -crystallin by reacting with it [81]. It also protects the enzyme, glucose 6-phosphate dehydrogenase, against fructation [82].

It has been claimed that measurement of carboxymethyllysine (CML) and pentosidine to indicate inhibition of glycation is unreliable because they are products of an oxidation step following the initial glycation [83]. They investigated the metal chelating ability of many of the inhibitors discussed above. Unfortunately their only assay of metal chelation was based on the copper catalysed oxidation of ascorbate, which was measured by the absorbance at 244nm, and many of the inhibitors have just the type of structure to react with ascorbate and yield a product absorbing even more strongly than ascorbate at 244 nm. Dilazep, an antiplatelet agent, also inhibits AGE formation and scavenges hydroxyl radicals [84]. However 50% of the maximum radical scavenging activity was achieved by a concentration that had no significant effect on AGE so the two properties may not be related.

In a comparison of a variety of reported inhibitors, binding of radiolabelled lysine to protein was inhibited most effectively by thiamine pyrophosphate, semicarbazide, cysteine, sodium metabisulphite, 3-mercaptopropionate, o-phenylenediamine and aminoguanidine [85]. The last four also inhibited crosslinking of protein by threose, and the concomitant increase in non-tryptophan fluorescence, and glycation by glucose.

3.2. Reversal of glycation

There have been two attempts to deglycate proteins. The first was by isolation of amadoriases, fructosylamine oxidases, from soil organisms and other microorganisms, but in the event these were only active against small substrates and did not deglycate proteins [18]. The second, fructosamine 3-kinase, is more

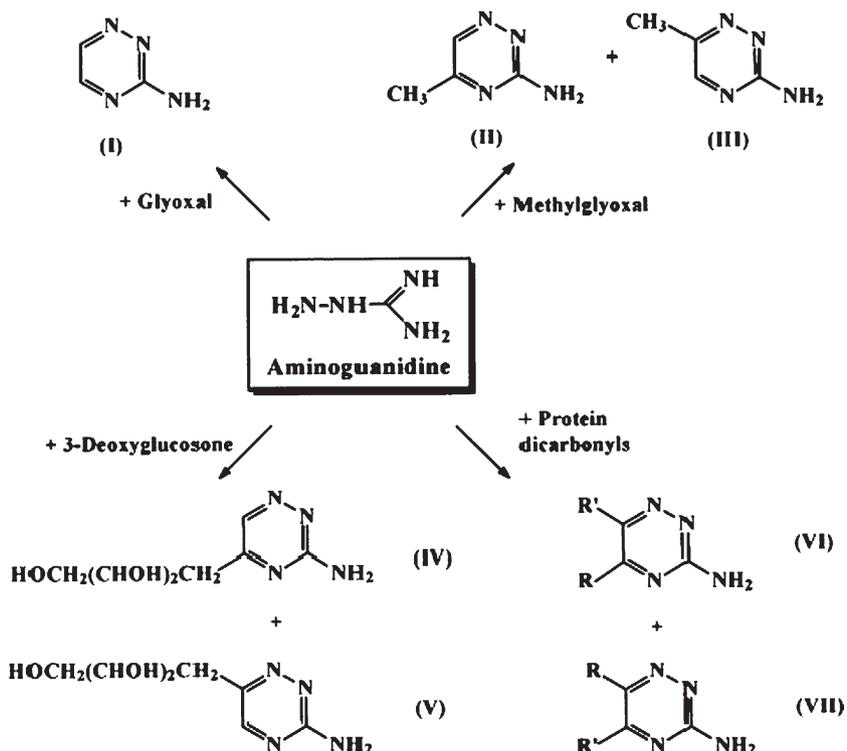


Fig. 4. The reaction of aminoguanidine with dicarbonyl compounds [61].

promising. It was first identified by Szwergold and then characterized as far as full sequence determination by two groups [86,87]. It acts by phosphorylating the bound sugar which then becomes unstable releasing 3-deoxyglucosone and restoring the original amino group. Thus the second step in the glycation pathway, previously regarded as largely irreversible, may be reversible by means of an enzymic reaction. The phosphorylation is not very specific at least with haemoglobin, but alpha-amino groups were not easily deglycated [88]. A deglycating pathway would explain why glycation sites in vivo differ from those identified in vitro. A second deglycating enzyme has been identified and both are assumed to play an important role in minimizing the modification of proteins in vivo [89]. Noting that spermine is a particularly good antiglycation agent of type b (Fig. 3) it has been proposed that fructosamine-3-kinase would then constitute a useful system to restore the spermine [90]. The enzyme has a high affinity for fructose-polyamine adducts and this adds to the plausibility of this notion, but the release of 3-deoxyglucosone (3-DG), a powerful glycating agent, close to an amino group of the protein remains a problem, although there are other enzymes to detoxify 3-DG [91]. Polymorphism in the fructosamine-3-kinase gene could affect glycation at specific sites on proteins [92].

Although the emphasis in prevention of glycation has been on mammalian systems, related enzymes are found in a variety of organisms and may serve to repair plant proteins subject to high concentrations of the powerful glycating sugars ribose 5-phosphate and erythrose 4-phosphate [93].

Szwergold [94,95] however suggests that in addition to these enzymic deglycation pathways there must be other natural deglycation strategies to explain several anomalies notably that insects lack fructosamine-3-kinase, and that birds cope with high glucose levels. It was known from the start that the first glycation step to form the Schiff base is reversible [1] but now Szwergold suggests that the reverse reaction, deglycation, depends on various intracellular nucleophiles including free amino acids and peptides such as glutathione, carnosine and anserine. These have already been proposed as preventing glycation (see above Section 2.1) but perhaps they also deglycate. The nucleophilic reversal of Schiff base formation had been suggested some years earlier [58]. These reactions could be important in birds that survive well with higher glucose concentrations and temperatures than mammals without ‘diabetic’ complications [94–96]. Szwergold showed by NMR how effectively glycine, taurine, spermidine and N-acetylcysteine but especially glutathione, other aminothiolumines, carnosine and anserine could eliminate a model Schiff base generating adducts with themselves. The role of glutathione is of particular interest as it is invariably regarded primarily as an antioxidant and is of great importance in the ocular lens, decreasing in almost all types of cataract, although the lens has almost negligible oxygen levels and no obvious oxidative hazard [97]. Insects cleverly have high levels of free amino acids and peptides and use trehalose, a non-reducing sugar, in the place of glucose as the predominant sugar [93]. It should be remembered that trehalose has other interesting properties serving as a chemical

chaperone or SOS molecule (see Section 3.4). It is possible that some of the benefits of nucleophiles attributed to prevention of glycation (Section 3.1) could be the result of this deglycation/transglycation.

3.3. Crosslink breakers

A decade ago the notion that the major AGE crosslink retained a dicarbonyl structure stimulated the development of a novel approach to diabetic complications with the design and synthesis of a range of crosslink breakers starting with *N*-phenacylthiazolium bromide (PTB) [98]. Unfortunately they have not lived up to their initial promise. PTB was first tested against AGE-crosslinked serum albumin that had been allowed to crosslink to a collagen matrix. The albumin was released. PTB also decreased the crosslinking of rat tail tendon collagen from diabetic rats, and disaggregated amyloid fibrils. In vivo it decreased the binding of IgG to erythrocytes [98]. One attraction of these results was that they supported the idea of a new type of glycation crosslink that was not fluorescent. There had been a paradox that all the identified crosslink compounds were present in tiny amounts and could not account for all the crosslinking apparent from physical studies.

PTB proved to be rapidly hydrolysed in water and the hydrolysis products could reduce disulphide crosslinks: potentially this could explain the apparent decreased protein crosslinking [99]. Research moved on to the more stable 4,5-dimethylthiazolium derivative of PTB, PMT usually called ALT-711. This compound improved arterial elasticity in diabetic rats without lowering blood glucose or glycated haemoglobin [100]. It decreased the excess crosslinking of collagen and of IgG to erythrocytes. Others, while agreeing that ALT-711 cleaves model dicarbonyls claim that it does not decrease collagen crosslinking in diabetic rats [101]. Yet others find the apparent decrease in collagen crosslinking can be achieved by a variety of compounds including metformin (see above) and 5-aminosalicylic acid [65]. ALT-711 prevented the loss of systolic function, and aortic stiffness found in diabetic dogs [102]. Early results on arterial elasticity from clinical trials were promising [103]. ALT-711, now called alagebrium, and PTB would not be expected to cleave the well known AGE crosslinks such as MOLD, GOLD, glucosepane and pentosidine. The controversy around this approach will undoubtedly be resolved by further clinical trials such as those described on the Alteon website (www.alteon.com).

3.4. Preventing the consequences of glycation

Crosslinking of proteins is one consequence of glycation and compounds to split crosslinks are being investigated (Section 3.3). Here we consider whether it is possible to prevent other consequences of glycation without necessarily preventing the glycation itself. The other consequences are in particular conformational change and aggregation leading to loss of function, oxidation and up-regulation of receptors for AGEs (RAGEs).

This possibility of protection against conformational change has been studied with enzymes where the loss of function is easily assessed. Alpha-crystallin, a molecular chaperone, is able to protect almost completely a variety of enzymes against glycation-induced inactivation *in vitro* without interfering with the original glycation reaction (Fig. 5) [31,104,105]. Even in the more physiological environment of the red cell ghost alpha-crystallin was able to protect eight different enzymes against inactivation by glycation with fructose [106]. Alpha-crystallin not only protects against enzyme inactivation it can also help to restore the structure and activity of enzymes such as glyceraldehyde -3-phosphate dehydrogenase [107].

Some small organic molecules share some protective properties with molecular chaperones and are often referred to as ‘chemical chaperones’ although a better term is ‘Small Organic Stress Molecules’ or ‘SOS molecules’ [108]. Of these, trehalose and 6-aminohexanoic acid protected glucose-6-phosphate dehydrogenase against glycation-induced inactivation. These compounds also helped to restore the activity of previously inactivated enzyme [108].

Glycation can lead to autoxidation, and anti-glycation compounds, aspirin, D-penicillamine and vitamin E, showed protective effects against high glucose and advanced glycation endproduct (AGE) mediated toxicity in cultured bovine aortic

endothelial cells [109]. All three compounds protected against the anti-proliferative effects of high glucose and BSA-AGE, with vitamin E being the most effective. These authors suggest that compounds, such as vitamin E, with combined antiglycation and antioxidant properties offer maximum therapeutic potential in protection against high glucose and AGE-mediated cellular toxicity. Of course glutathione not only prevents glycation (Section 2.1) but also serves as an anti-oxidant as do other thiols.

The thiol group of cysteine, a powerful nucleophile can react with dicarbonyl compounds to give thiol-aldehyde adducts, in the case of glyoxal, S-(carboxymethyl) cysteine is formed [110]. The process is accompanied by loss of the thiol group and formation of stable products. Other cysteine compounds are effective against glycation effects whereas diallyl sulphide and disulphide were more effective anti-oxidants [111]. Sodium sulfite is an antioxidant and suppressed methylglyoxal-induced hypervascularity and AGE formation in the peritoneum, as well as macroscopic alterations in rats [112].

Minodronate, a nitrogen-containing bisphosphonate, blocked the angiogenic signalling of vascular endothelial growth factor in endothelial cells through its antioxidative properties. It completely inhibited the AGE-induced ROS generation in human umbilical vein endothelial cells by suppressing NADPH oxidase-derived ROS generation, probably via inhibition of

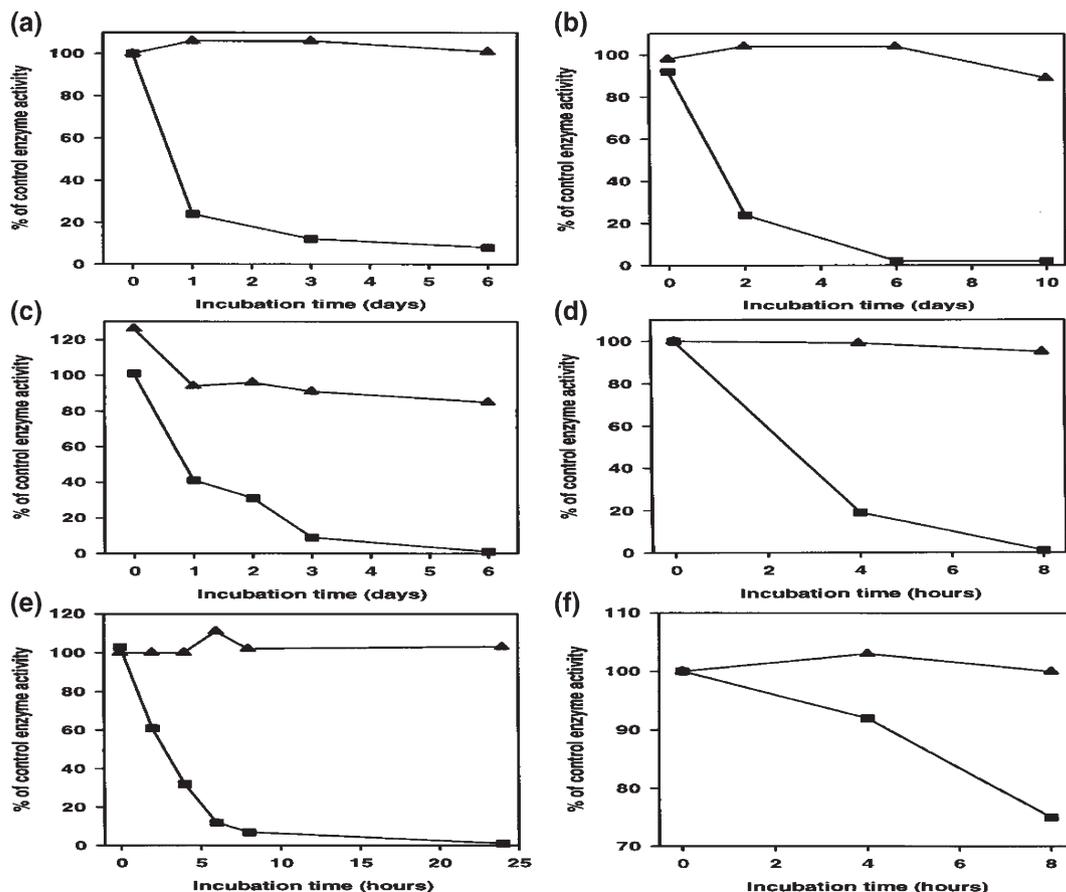


Fig. 5. Protection by α -crystallin against the fructose-induced inactivation of (a) glutathione reductase, (b) catalase, (c) superoxide dismutase, (d) glucose 6-phosphate dehydrogenase, (e) Malate dehydrogenase, (f) fumarase. Taken from Hook and Harding [104].

geranylgeranylation of Rac, a component of endothelial NADPH oxidase [113]. Tetrahydrocurcumin (THU1), administered to 25% galactose-fed SD rats showed that scavenger ROS not only formed during hyperglycemia, but also induced anti-oxidative enzymes such as glutathione S-transferase. THU1 also showed a significant increase of glutathione (GSH) concentration in the cultured rat lens [114].

There are data suggesting that thiamine and benfotiamine (S-benzoylthiamine monophosphate), a vitamin B1 derivative, can prevent or alleviate some of diabetic complications, such as nephropathy [115], microangiopathy [116], and retinopathy [117]. One of the effects of thiamine and benfotiamine is to reduce the formation of AGE compounds. However, the beneficial effects of benfotiamine, such as the decrease of diabetes-induced cerebral oxidative damage [118], or antagonizing STZ-induced cardiac contractile dysfunction in mouse cardiomyocytes [119] may involve mechanisms other than AGE formation. Some AGE compounds, (CML and CEL) in diabetic rats, are not normalized by benfotiamine [120].

RAGE is a member of immunoglobulin superfamily of cell surface molecules; its interaction with ligands enhances receptor expression. The blockade of RAGE expression may have a therapeutic effect.

Nifedipine, a well-known calcium antagonist inhibited RAGE over expression in AGE-exposed endothelial cells by suppressing ROS generation [121].

Telmisartan, an Angiotensin II type 1 receptor antagonist blocked completely both Ang II up-regulation of RAGE mRNA levels of microvascular epithelial cells and the subsequently increased soluble form of RAGE (sRAGE) expression in the medium [122].

4. Protection against other post-translational modifications of proteins

Proteins are attacked by many small molecules other than sugars *in vivo* with equally damaging consequences. These reactions have received less attention as have the ways to prevent them, but other small molecules can cause conformational changes and inactivation of enzymes (Section 2.2). The damaging small molecules include cyanate, aldehydes, ketones, and steroids [1].

Aspirin and ibuprofen protect proteins against reaction with cyanate and prednisolone [97]. They also protect incubated rat lenses against cyanate-induced opacification [25,29]. The chaperone alpha-crystallin protected enzymes against inactivation by both steroids and malondialdehyde [123,124].

Even with the limited attention received it is clear that other uncontrolled nonenzymic post-translational modifications have damaging effects on proteins and that the means for protection are similar. Most of the modifications involve an electrophilic attack on vulnerable nucleophiles on the proteins such as amino and thiol groups. Some of the protecting agents have been presented as competing nucleophiles. Very recently Szwergold [94–96] proposed, in relation to glycation, that the protective nucleophiles may not simply compete for the damaging sugars (electrophiles) but that they could deglycate by a transglycation

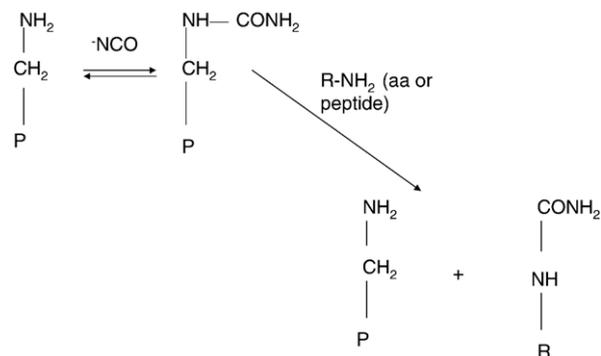


Fig. 6. Transcarbamylation protecting against the effects of high levels of cyanate. The initial reaction of cyanate (isocyanate) is reversed by an amino acid or peptide.

action. He proposed that this deglycation system was more important than the enzyme systems discussed in Section 3.2 because the chemical system acts at an earlier stage, and proposed that free amino acids and peptides including glutathione would be active in this role. I would propose that the nucleophilic system is even more important because it could deal with other electrophiles such as cyanate, corticosteroids, aldehydes and ketones. For example, small nucleophiles could reverse carbamylation, which has been implicated in the sequelae of renal failure and even of severe diarrhoea. The attacking cyanate comes from the equilibrium with urea. It has been a puzzle why cartilaginous fish seem unharmed by high plasma urea levels but it was pointed out that they also had high levels of amines [125]. Perhaps the amines do more than protect against carbamylation. They could also reverse carbamylation (Fig. 6).

5. Conclusions

It is clear that glycation is responsible for many of the sequelae of diabetes, and for some changes in ageing, and therefore prevention of glycation or protection against the consequences of glycation could be beneficial for millions of people. Similarly there is evidence for the harmful effects of other non-enzymic, post-translational modifications of proteins. Fortunately a variety of compounds have been studied *in vitro* for their possible benefits, and some *in vivo*. Although potential hazards exist many of the compounds are either well established or natural products which might be expected to do more good than harm.

It is more difficult to see how the *in vitro* benefits of deglycating enzymes and molecular chaperones could transfer towards therapy.

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