Amino Acids (2012) 42:1143–1150
DOI 10.1007/s00726-010-0780-3

REVIEW ARTICLE

Enzymatic repair of Amadori products

Emile Van Schaftingen · François Collard · Elsa Wiame · Maria Veiga-da-Cunha

Received: 7 June 2010 / Accepted: 22 August 2010 / Published online: 22 October 2010

Abstract Protein deglycation, a new form of protein repair, involves several enzymes. Fructosamine-3-kinase (FN3K), an enzyme found in mammals and birds, phosphorylates fructosamines on the third carbon of their sugar moiety, making them unstable and causing them to detach from proteins. This enzyme acts particularly well on fructose-epsilon-lysine, both in free form and in the accessible regions of proteins. Mice deficient in FN3K accumulate protein-bound fructosamines and free fructoselysine, indicating that the deglycation mechanism initiated by FN3K is operative in vivo. Mammals and birds also have an enzyme designated 'FN3K-related protein' (FN3KRP), which shares ≈65% sequence identity with FN3K. Unlike FN3K, FN3KRP does not phosphorylate fructosamines, but acts on ribulosamines and erythrulosamines. As with FN3K, the third carbon is phosphorylated and this leads to destabilization of the ketoamines. Experiments with intact erythrocytes indicate that FN3KRP is also a protein-repair enzyme. Its physiological substrates are most likely formed from ribose 5-phosphate and erythrose 4-phosphate, which give rise to ketoamine 5- or 4-phosphates. The latter are dephosphorylated by 'low-molecular-weight protein-tyrosine-phosphatase-A' (LMW-PTP-A) before FN3KRP transfers a phosphate on the third carbon. The specificity of FN3K homologues present in plants and bacteria is similar to that of mammalian FN3KRP, suggesting that deglycation of ribulosamines and/or erythrulosamines is an ancient mechanism. Mammalian cells contain also a phosphatase acting on fructosamine 6-phosphates, which result from the reaction of proteins with glucose 6-phosphate.

Keywords Deglycation · Fructose-epsilon-lysine · Fructosamine · Ribulosamine · Fructosamine-3-kinase · Protein repair

E. Van Schaftingen (✉) · F. Collard · E. Wiame · M. Veiga-da-Cunha
de Duve Institute, Université catholique de Louvain, Avenue Hippocrate 75, B-1200 Brussels, Belgium
e-mail: emile.vanschaftingen@uclouvain.be

Protein repair versus DNA repair

Repair is of outstanding importance for life. All biomolecules get altered with time because of chemical instability, aggression by 'chemicals' or inappropriate modification by enzymes, and in many cases, repair mechanisms exist. This is particularly true not only for DNA, but also for proteins and even for metabolites (Galperin et al. 2006; Van Schaftingen et al. 2009). Before deglycation, the subject of the present review, two other protein repair mechanisms have been described: the reduction of protein-methionine sulphoxide residues by methionine sulphoxide reductases (Moskovitz 2005) and the conversion of isoaspartyl residues (which spontaneously arise from asparagines or aspartates) to L-aspartates by protein-L-isoaspartate methyltransferase (PIMT; Clarke 2003). Unlike DNA, which is a rather accessible molecule, the folding of proteins makes that a large share of their amino acid residues are not accessible to enzymes—not only repair enzymes, but also modifying enzymes, such as protein kinases. This is the reason why protein repair is incomplete.

The reason why protein repair is important is not known presently. The fact that mice deficient in PIMT show major neurological problems (Kim et al. 1997) argues for the

importance of protein repair. A common feature of protein repair mechanisms is that they require energy, in the form of reducing equivalents (methionine sulphoxide reductases), S-adenosylmethionine (PIMT), or ATP (deglycation; see below). This is not surprising considering that reversing a spontaneous process is likely to require energy, but this has also a consequence, which is that protein repair mechanisms are limited to where energy is available, the cell interior.

Important glycating agents under physiological conditions

Protein glycation is a spontaneous reaction of reducing sugars with amino groups of proteins. It starts with the formation of a Schiff base, which slowly undergoes an Amadori rearrangement to become a ketoamine (Hodge 1955; Baynes et al. 1989). Fructosamines are formed when glucose is the reacting sugar, ribulosamines and erythrulosamines when the starting sugar is ribose or erythrose. These early glycation products get spontaneously converted to advanced glycation endproducts (AGEs), but until now no repair mechanism for these has been described. Glycation with aldoses takes place with the epsilon-amine of lysines and the N-terminal amino group of proteins if it is free. It takes place in vivo: a significant proportion of haemoglobin—about 5% of total haemoglobin in normoglycemic subjects—has a 'fructosamine' bound to the amino-terminus of its beta chains (HbA1c) or to a lysine side-chain.

Glucose is the most abundant free aldose in vertebrate tissues and it is therefore the most important glycating agent in vivo. However, it is intrinsically much less reactive than other physiological sugars such as glucose 6-phosphate, ribose, ribose 5-phosphate and erythrose 4-phosphate, which are respectively about 5-, 10-, 100- and 400-fold more reactive (Fortpied et al. 2006; Gemayel et al. 2007). The reason for this difference is that the reactivity of sugars depends on the proportion under which they exist with a free carbonyl group. This proportion is very low in the case of glucose, which is essentially (>99.9%) under its hemiacetal forms in solution. Other sugars or sugar phosphates exist to a larger extent (e.g. ribose, ribose 5-phosphate) or even completely (erythrose 4-phosphate) in linear form, which increases their reactivity. The presence of a phosphate group also increases reactivity, presumably because it functions as a local acid–base catalyst. This means that despite their lower intracellular concentrations, aldose-phosphates may be as significant glycating agents as glucose in higher vertebrates and that they are much more important than glucose in most other organisms.

Mechanisms of deglycation

Ketoamines are imines and detaching them from proteins or amino acids implies that a carbon–nitrogen single bond has to be severed. This is apparently not simple and the strategy that has been invariably used by Nature to cut these bonds is to convert them first to N=C double bonds, i.e. Schiff bases, which are easily hydrolysed. Three different types of enzymatic mechanisms are known to metabolize ketoamines: they involve oxidases, isomerases and kinases (Fig. 1). Only the latter is used to deglycate proteins under physiological conditions.

Amadoriases are microbial (mostly fungal) oxidases, which, as a rule, catalyze the oxidation between C1 of the sugar portion and the nitrogen atom, releasing the dicarbonyl compound glucosone when they act on a fructosamine (reviewed by Monnier and Wu 2003). These enzymes are distantly related to sarcosine oxidases, use FAD as a cofactor and reduce O_2 to H_2O_2. The reaction they catalyze is therefore irreversible. Not surprisingly, the eukaryotic members appear to be located in peroxisomes. Amadoriases have been initially identified because they allow growth of different microbes on low-molecular-weight fructosamines and related ketoamines. These enzymes act very well on low-molecular-weight compounds, but much less so on fructosamines bound to peptides or to proteins. The crystal structure of one of these amadoriases (Collard et al. 2008) shows that the catalytic site is in a deep crevice, explaining that only low-molecular-mass substrates have a good accessibility and are metabolized by these enzymes.

A few isomerases, called 'deglycases' and catalyzing a 'reverse Amadori rearrangement', are involved in the metabolism of fructosamines in bacteria (Wiame et al. 2002, 2004, 2005). Fructosamines are first phosphorylated to fructosamine 6-phosphates, which are then converted by deglycases to glucose 6-phosphate and a free amino acid, most probably through an intermediary Schiff base. The phosphorylation involves either an ATP-dependent kinase or a phosphotransferase system (PTS), which transports its substrate and phosphorylates it at the expense of phosphoenolpyruvate. It is not known if the deglycases act on protein-bound fructoselysine 6-phosphates, but this is most likely not the case. Whatever the answer to this question, they catalyze easily reversible reactions and for this reason, are not suited to catalyze protein deglycation.

The last class of enzymatic reactions leading to deglycation involves kinases that phosphorylate the third carbon of the sugar moiety of ketoamines. This leads to their destabilization and their separation from the amino groups as 2-keto-3-deoxyaldoses. This second reaction is spontaneous and it is much faster with erythrulosamine 3-phosphates than with ribulosamine 3-phosphates or fructosamine

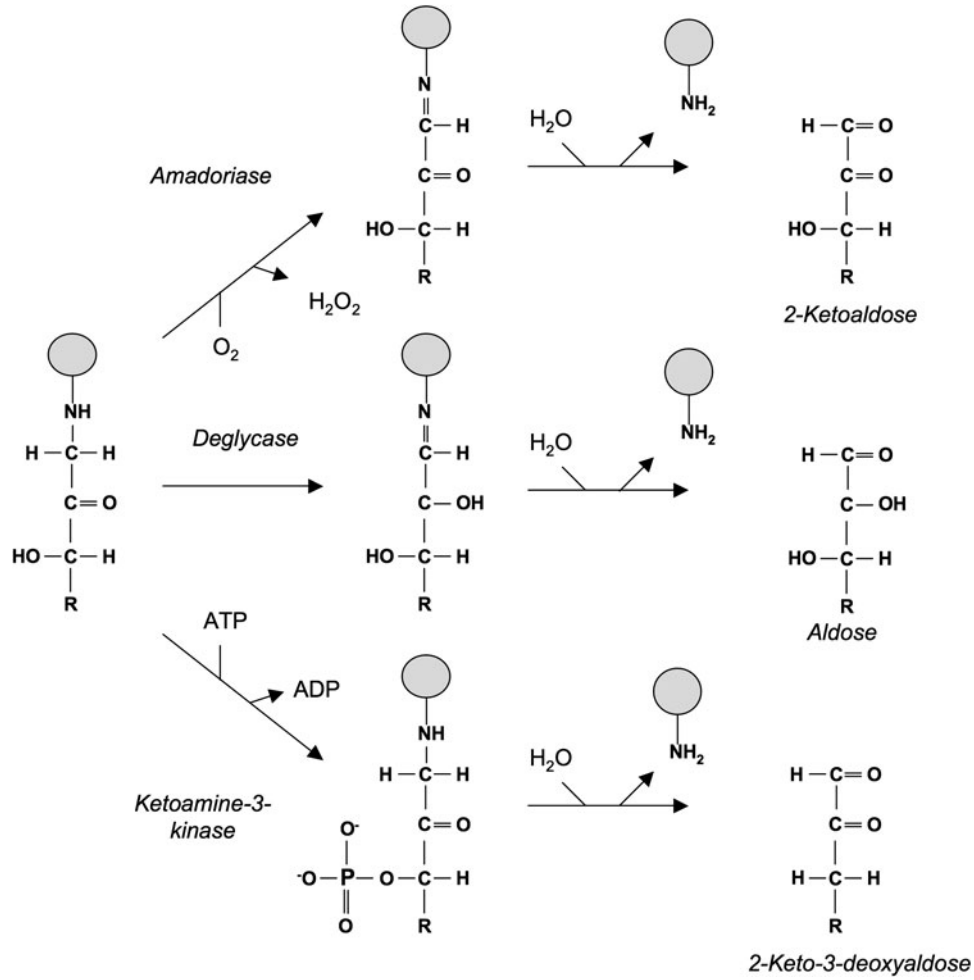

Fig. 1 Enzymatic reactions involved in the metabolism of ketoamines. Ketoamines (e.g. fructosamines) are metabolized oxidatively by amadoriases, leading to the formation of a 2-ketoaldose (e.g. glucosone). Deglycases catalyze a 'reverse Amadori reaction' with fructosamine 6-phosphates as substrates, leading to the formation of an aldose (e.g. glucose 6-phosphate). Ketoamine-3-kinases phosphorylate the third carbon of the sugar portion of a ketoamine, causing their destabilization and the formation of a 2-keto-3-deoxyaldose (e.g. 3-deoxyglucosone). The amino group carrier (*circle*) may be a protein or a low-molecular-weight compound

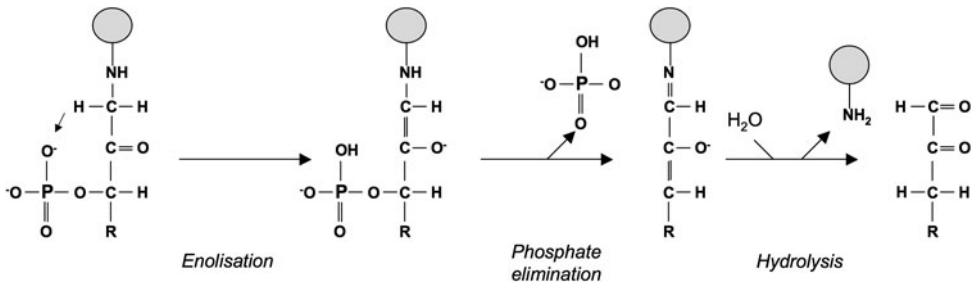

Fig. 2 Hypothetical mechanism for the breakdown of ketoamine 3-phosphates. The phosphate group in C3 presumably plays the role of an acid–base catalyst, facilitating the enolisation of the substrate. Its β-elimination leads to the formation of a Schiff base, which is then hydrolysed

3-phosphates (half-lives of <5 min, 20 min and 8 h, respectively, at neutral pH and 37°C). This suggests that deglycation proceeds from the open-chain form of ketoamine 3-phosphates. The mechanism of the effect of the phosphate group has not been investigated in detail, but it is likely that this group acts as an acid–base catalyst that facilitates the deprotonation of carbon 1 (Fig. 2). Furthermore, phosphate is a much better leaving group than OH^-. The proposed mechanism is analogous to the one involving the generation of methylglyoxal from triose phosphates (Phillips and Thornalley 1993; Richards 1993). The overall process of deglycation initiated by ketoamine-3-kinases consumes ATP, which makes it irreversible. The enzymes that catalyze the phosphorylation of fructosamines and related compounds belong to the same family as protein kinases, and it is therefore not surprising that they are able to act on protein substrates.

Fructosamine-3-kinase (FN3K), the prototypic deglycation enzyme

Discovery

The discovery of fructose 3-phosphate in human and animal tissues (Szwergold et al. 1990, Petersen et al. 1990)

was the starting point for the discovery of fructosamine-3-kinase. This phosphate ester had no known function and investigations on the mechanism of its synthesis in erythrocyte extracts showed that an ATP-dependent kinase was involved, but that this enzyme had a very low affinity ($K_M \geq 30$ mM for its substrate) and a low metabolic capacity (Petersen et al. 1992). These findings suggested that the putative 'fructose-3-kinase' acted on something different than fructose, though most likely structurally related to it. Independent work in Szwergold's group (Szwergold et al. 2001) and in our group (Delpierre et al. 2000) led to the conclusion that fructose-3-kinase phosphorylates fructosamines with a K_M in the micromolar range, i.e. 4–5 orders of magnitude lower than the K_M for fructose ($\approx$50–100 mM). Once a good substrate was found, the enzyme could be easily purified from human erythrocytes and its cDNA was cloned (Delpierre et al. 2000; Szwergold et al. 2001).

Specificity, properties

Human FN3K is a 309 amino acid monomeric protein, distantly related to aminoglycoside kinases and, even more distantly, to protein kinases. Its N-terminal methionine is conserved in the mature protein and N-acetylated (Delpierre et al. 2000). It has been purified to near-homogeneity from human erythrocytes and has been produced as a recombinant protein in bacteria. FN3K phosphorylates not only fructosamines, but also their C3-epimers psicosamines (=allulosamines), as well as ribulosamines and erythrulosamines. The C3 carbon in the latter three types of substrates has the opposite configuration compared with fructosamines, yet the oxygen borne by this carbon appears to be in all cases the phosphoryl acceptor. It should be noted that psicosamines (which are not physiological) are much poorer substrates than the other types of ketoamines.

The nature of the aglycone is also important: the enzyme displays a K_M in the micromolar range with fructose-epsilon-lysine and deoxymorpholinofructose (a non-physiological, but cell-permeable substrate), and of the order of 1 mM for fructosevaline, fructoseglycine or fructoseglycylglycine, indicating that the presence of an alpha-carboxylic group near the glycated amine negatively affects binding to the catalytic site. In vitro studies on the phosphorylation of fructosamines bound to haemoglobin indicate that several fructosamines bound to lysines are excellent substrates, whereas others are only poorly phosphorylated (Delpierre et al. 2004). Thus, the fructosamines bound to Lys139α (near the C-terminus of the α subunits) and Lys16α (on a loop of the same subunits) are good substrates, whereas that bound to Lys61α, whose side chain is partially bound to a heme, is only very slowly phosphorylated. This suggests that polypeptide regions surrounding the glycated lysine must be flexible enough to enter the catalytic site of FN3K. The N-terminal glycated valine is a poor substrate, consistent with free fructosevaline being a much poorer substrate than free fructoselysine.

Tissue and species distribution

FN3K is present in mammals and birds, but not in fishes, plants or bacteria. The existence of an orthologue in xenopus suggests that it is also present in amphibians. In mouse and rat tissues, FN3K activity is present in almost all tissues but is particularly elevated in brain, kidneys and red blood cells. Erythrocytes from pig and chicken, two species in which the intracellular glucose concentration is low, have very low FN3K activity whereas high activities are found in erythrocytes from species (human, mouse, rat) in which the intra-erythrocyte glucose concentration is equal to that of the plasma. This finding is consistent with FN3K being involved in the removal of fructosamines. The activity of FN3K and the amount of its mRNA in different tissues are independent from starvation (Delplanque et al. 2004). No regulation of the expression of FN3K was observed in human fibroblasts treated with conditions mimicking the hormonal and biochemical profile of the diabetic state (Conner et al. 2004).

Function in deglycation

The proof that FN3K is indeed responsible for deglycation was first provided by the finding that deoxymorpholinofructose (DMF), a competitive inhibitor (and also a substrate) of FN3K, increased about twofold the rate of accumulation of glycated haemoglobin when erythrocytes are incubated in the presence of 200 mM glucose (Delpierre et al. 2002). This difference is only twofold because inhibition of FN3K exclusively affects the glycation of lysine residues that are accessible to this enzyme, the other positions being 'insensitive' to FN3K activity. This interpretation could be confirmed by the identification of the glycation sites in both situations (Delpierre et al. 2004).

Definitive evidence for FN3K being responsible for deglycation was provided by showing that the level of haemoglobin-bound fructosamines is about 2.5-fold higher in $FN3K^{-/-}$ mice than in $FN3K^{+/+}$ or $FN3K^{+/-}$ mice. Similarly, cytosolic proteins are 1.5- to 1.8-fold more glycated in liver, kidney, brain and sketetal muscle of $FN3K^{-/-}$ mice than of $FN3K^{+/+}$ mice (Veiga-da-Cunha et al. 2006). The concentration of free fructoselysine was also found to be increased by up to $\approx$10-fold, indicating that FN3K also acts in vivo on low-molecular-weight compounds. No consistent pathological findings have, however, been observed with the $FN3K^{-/-}$ mice. Furthermore, lack of FN3K activity did not appear to enhance

mortality in streptozotocin-diabetic mice, despite the fact that FN3K$^{-/-}$ mice showed very high levels of glycated haemoglobin ($\approx$30%) (Veiga-da-Cunha, unpublished results). Absence of FN3K does also not affect the functioning of pancreatic β-cells, even if these are incubated for several weeks in the presence of elevated glucose concentrations (Pascal et al. 2010).

Variation of FN3K activity in humans

The human erythrocyte FN3K activity, which is stable with time in a single individual, is highly variable from person to person. This variability, which is linked to SNPs that are present in the FN3K gene, affects the glycation level of at least one specific site in haemoglobin (Delpierre et al. 2004). A recent study (Mohás et al. 2010) involving $\approx$850 type II diabetic patients showed that diabetic subjects with the CC variant of SNP rs1056534 (G900C), which is associated with a higher FN3K activity, had lower HbA1c levels compared with other genotypes. No association between CC genotype and diabetic complications, such as diabetic nephropathy, neuropathy and retinopathy could be found. These findings indicate that the variability in FN3K activity may provide an explanation for the 'glycation gap', i.e. the fact that the glycation of haemoglobin does not perfectly correlate with the mean glucose level (Leslie and Cohen 2009). It would be interesting to expand these studies and try and correlate the FN3K activity with the glycation gap and with the development of diabetic complications in larger series of patients. The FN3K assay proposed by Krause et al. (2006) could be useful in this respect.

FN3K-related-protein, a ribulosamine/erythrulosamine-3-kinase

FN3K-related protein (FN3KRP) shares 65% sequence identity with FN3K (Collard et al. 2003). The genes encoding both proteins are in tandem repeat, indicating that they result from a gene duplication event. Only one copy of the gene is found in fishes and in more primitive organisms, including bacteria, whereas two copies are found in birds and in mammals, indicating that the gene duplication event occurred during fish radiation. Surprisingly, mammalian and bird FN3KRPs do not phosphorylate fructosamines, but they act on ribulosamines, erythrulosamines and, with a lower affinity, psicosamines (Collard et al. 2003; Payne et al. 2008). The K_M value for ribuloselysine is $\approx$2 μM. All these substrates appear to be phosphorylated on their third carbon (Collard et al. 2003; Delplanque et al. 2004; Fortpied et al. 2005). Thus unlike FN3K, which phosphorylates C_3 either in the L- or the D-configuration, FN3KRP is specific for C_3 in the D-configuration.

Szwergold et al. (2007) showed that FN3KRP phosphorylates meglucamine and other sorbitolamines on their 4th carbon. The phosphorylated carbon has a D-configuration, whereas the 3rd carbon of sorbitolamines has an L-configuration, in agreement with the strict stereospecificity of FN3KRP. These reactions have been studied with elevated concentrations of substrate (10 mM) and no K_M value is reported. Since polyolamines are unlikely to be present under physiological conditions, this finding does not argue against ribulosamines and erythrulosamines being the best substrates and most probably the physiologically relevant ones. The finding that the distance between the amino group and the phosphorylated oxygen may be somewhat longer than in ribulosamines may be a clue to the identification of additional substrates.

Since ketoamine 3-phosphates are unstable (see above), FN3KRP has also the potential of being a protein-deglycating enzyme. That it can play this role in intact cells was demonstrated with erythrocytes incubated with ribose or allose, to generate ribulosamines and psicosamines, respectively: inhibition of both FN3K and FN3KRP increased the accumulation of haemoglobin-bound ribulosamines and psicosamines (Collard et al. 2004), whereas an inhibitor of FN3K was unable to block this deglycation on its own.

The potential importance of ribulosamines (and most likely also erythrulosamines, which have been less systematically studied because of their instability) as substrates of FN3KRP is indicated by the finding that they are substrates for the fish and plant homologues of FN3K/FN3KRP, as well as for most bacterial homologues that have been tested (Fortpied et al. 2005; Gemayel et al. 2007), whereas fructosamines are not, or only very poor substrates for these enzymes. These findings indicate that the ancestral protein was a ribulosamine/erythrulosamine-3-kinase. Intriguingly, no activity could be found on any ketoamine substrates with the *Escherichia coli* and *Salmonella typhimurium* FN3K homologues.

A phosphatase acting on ribulosamine 5-phosphates

Ribulosamines and erythrulosamines do probably not form from free ribose or free erythrose, because these compounds are not expected to be present in free form inside cells. A more likely possibility is that they arise from the pentose-phosphate-pathway intermediates, ribose 5-phosphate and erythrose 4-phosphate, which are extremely powerful glycating agents (see above). However, FN3KRP and homologous proteins do not phosphorylate

ribulosamine 5-phosphates (and most likely also erythrulosamine 4-phosphates) on their third carbon, and a phosphatase is therefore needed to convert ribulosamine 5-phosphates and erythrulosamine 4-phosphates to substrates of FN3KRP (Fig. 3).

Consistent with this hypothesis, a phosphatase that catalyzes the dephosphorylation of ribulosamine 5-phosphates has been purified from human erythrocytes and identified as 'low-molecular-weight protein-tyrosine-phosphatase A' (LMW-PTP-A) (Fortpied et al. 2007). The ribulosamine-5-phosphatase activity of LMW-PTP-A is higher than its protein-tyrosine-phosphatase activity. Furthermore, several bacterial genomes contain an operon encoding both a LMW-PTP and a FN3K homologue, suggesting that LMW-PTP also serves to dephosphorylate ribulosamine 5-phosphates and/or erythrulosamine 4-phosphates in bacteria (Gemayel et al. 2007).

A phosphatase acting on fructosamine 6-phosphates

One of the surprising observations made on the FN3K$^{-/-}$ mice is that muscle cytosolic proteins are significantly more glycated than what one would expect from the cytosolic glucose level in skeletal muscle. The concentration of free glucose in muscle is much lower (probably less than 0.1 mM) than in liver cytosol (at least 5 mM), whereas the level of fructosamines bound to muscle cytosolic proteins is about half that found in liver (Veiga-da-Cunha et al. 2006). An explanation for this paradoxical observation is that glucose 6-phosphate is another important glycating agent: it is intrinsically more reactive than glucose (see above) and about as potent as this sugar if one takes into account that its intracellular concentration is of the order of 0.2–0.5 mM.

Fructosamine 6-phosphates, the direct product of glycation with glucose 6-phosphate, are not substrates for FN3K and again, as for ribulosamine 5-phosphates, a phosphatase is needed to convert them to fructosamines. Such a phosphatase was indeed detected in rat tissues, and identified as Magnesium-dependent phosphatase 1 (MDP-1) (Fortpied et al. 2006). This enzyme was till then known to act best on arabinose 5-phosphate, a non-physiological sugar-phosphate (Selengut and Levine 2000; Selengut 2001). Determination of its three-dimensional structure indicated that unlike other members of the large family of phosphatases to which it belongs (the HAD family), it lacks the 'cap' that closes the catalytic site in other enzymes of the same family (Peisach et al. 2004). This structural information suggested that MDP-1 was acting physiologically on macromolecular substrates, presumably proteins. We now know that its best substrates are protein-bound fructosamine 6-phosphates, for which the catalytic efficiency is about 20-fold higher than for free fructoselysine 6-phosphate or arabinose 5-phosphate, and about 100-fold higher than for a protein-tyrosine-phosphate substrate (Fortpied et al. 2006). MDP-1 therefore acts most likely in vivo as a fructoselysine-6-phosphate-phosphatase, but this remains to be proven by creating models of deficiency of this enzyme.

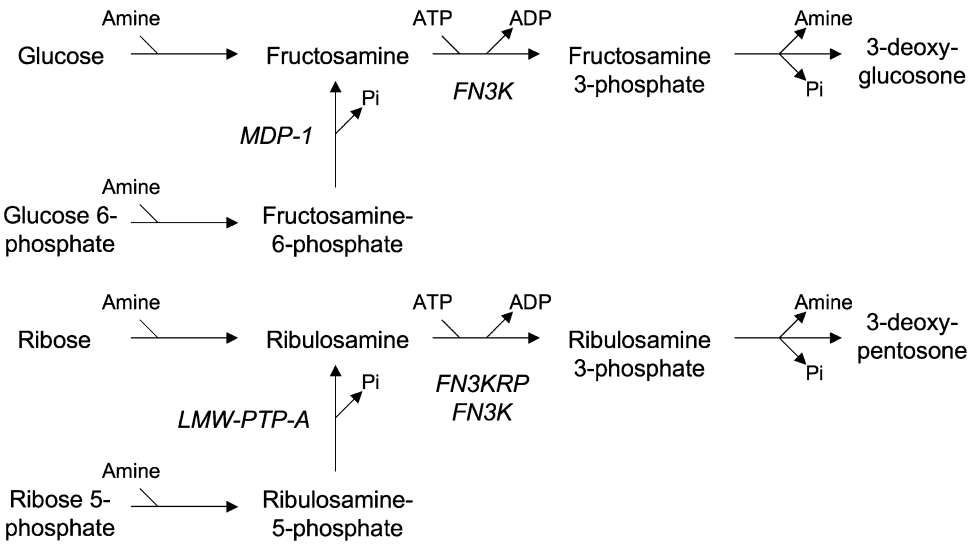

Fig. 3 Enzymes involved in deglycation. Fructosamines, fructosamine 6-phosphates, ribulosamines and ribulosamines 5-phosphate form spontaneously. Magnesium-dependent phosphatase 1 (MDP-1) dephosphorylates fructosamine 6-phosphates, whereas low-molecular-weight phosphotyrosine phosphatase (LMW-PTP-A) dephosphorylates ribulosamine 5-phosphates. FN3K-related protein (FN3KRP) phosphorylates ribulosamines and fructosamine-3-kinase (FN3K) fructosamines and ribulosamines. Erythrulosamine 4-phosphates are presumably formed from erythrose 4-phosphate and processed like ribulosamine 5-phosphates (not shown)

Other mechanisms for preventing or removing glycation adducts

Szwergold (2005) showed that the dipeptides carnosine and anserine, at physiologically relevant concentrations, promote the breakdown of the Schiff base glucosylethylamine by attacking these compounds and forming glucosylcarnosine and glucosylanserine, respectively. However, one may wonder if these dipeptides, which have a pK value in the physiological range, do not also facilitate the formation of Schiff bases and Amadori products from amines and glucose by playing the role of acid–base catalysts. It would therefore be interesting to verify the effect of these dipeptides on the accumulation of Amadori products in vivo. The recent cloning of carnosine synthase (Drozak et al. 2010) should facilitate such experiments.

Cysteine and other thiol-containing amines, particularly cysteamine also promote the decomposition of Schiff bases, leading to the irreversible formation of thiazolidines (Szwergold 2006). It should be noted that the enhancement of the rate of decomposition of the Schiff base is relatively small, particularly if one considers that the concentration of cysteine and cysteamine used (50 mM) is several orders of magnitude higher than their normal intracellular concentration. The physiological significance of these mechanisms remains to be established.

Conclusion and perspectives

The past 20 years have witnessed a revolution in our concepts on glycation: a process that was thought to be irreversible is now known to be corrected by intracellular enzymes. A lot of work needs to be done to elucidate the consequences of defects in deglycation, particularly in humans. The role of other enzymes like FN3KRP and the phosphatases that act on ketoamine 4-, 5- or 6-phosphates still needs to be assessed with knockout models. This is true also for low-molecular-weight antiglycating agents such as carnosine and thiolamines.

A challenge will be to identify the selective advantage that such protein repair mechanisms confer. Considering that glycation only affects a few percent of the cytosolic proteins, even in FN3K$^{-/-}$ mice, the perturbation caused by the absence of deglycation is most likely not due to the loss of the function of a protein, but most probably to the appearance of a new property. This could be, for instance, mistargeting through the modification of lysines present in a peroxisomal or nuclear targeting signal; abnormal degradation due to modification of a lysine involved in ubiquitin ligation; abnormal phosphorylation by a protein kinase, due to modification of a lysine present in a phosphorylation site consensus; abnormal regulation, due to modification of an acetylation site. The multiplicity of the potential mechanisms combined with the number of potential targets makes the task of identifying the proteins for which 'deglycation makes a difference' particularly hard.

The identification of enzymes working on glycation products made from ribose 5-phosphate and erythrose 4-phosphate and their widespread occurrence in Nature stresses also the importance of glycation by these agents. Understanding their spontaneous fate in vivo when deglycation is deficient would be most interesting.

Acknowledgments This work was supported by the Interuniversity Attraction Poles Program-Belgian Science Policy, by the Belgian Scientific Fund for Medical Research (FRSM), and by the DIANE centre of excellence programme of the Région Wallonne. FC and EW are Chargés de Recherche and MVDC is Chercheur Qualifié of the Fonds National de la Recherche Scientifique.

References

Baynes JW, Watkins NG, Fisher CI, Hull CJ, Patrick JS, Ahmed MU, Dunn JA, Thorpe SR (1989) The Amadori product on protein: structure and reactions. Prog Clin Biol Res 304:43–67

Clarke S (2003) Aging as war between chemical, biochemical processes: protein methylation, the recognition of age-damaged proteins for repair. Ageing Res Rev 2:263–285

Collard F, Delpierre G, Stroobant V, Matthijs G, Van Schaftingen E (2003) A mammalian protein homologous to fructosamine-3-kinase is a ketosamine-3-kinase acting on psicosamines and ribulosamines but not on fructosamines. Diabetes 52:2888–2895

Collard F, Wiame E, Bergans N, Fortpied J, Vertommen D, Vanstapel F, Delpierre G, Van Schaftingen E (2004) Fructosamine 3-kinase-related protein and deglycation in human erythrocytes. Biochem J 382:137–143

Collard F, Zhang J, Nemet I, Qanungo KR, Monnier VM, Yee VC (2008) Crystal structure of the deglycating enzyme fructosamine oxidase (amadoriase II). J Biol Chem 283:27007–27016

Conner JR, Beisswenger PJ, Szwergold BS (2004) The expression of the genes for fructosamine-3-kinase and fructosamine-3-kinase-related protein appears to be constitutive and unaffected by environmental signals. Biochem Biophys Res Commun 323:932–936

Delpierre G, Rider MH, Collard F, Stroobant V, Vanstapel F, Santos H, Van Schaftingen E (2000) Identification, cloning, and heterologous expression of a mammalian fructosamine-3-kinase. Diabetes 49:1627–1634

Delpierre G, Collard F, Fortpied J, Van Schaftingen E (2002) Fructosamine 3-kinase is involved in an intracellular deglycation pathway in human erythrocytes. Biochem J 365:801–808

Delpierre G, Vertommen D, Communi D, Rider MH, Van Schaftingen E (2004) Identification of fructosamine residues deglycated by fructosamine-3-kinase in human hemoglobin. J Biol Chem 279:27613–27620

Delplanque J, Delpierre G, Opperdoes FR, Van Schaftingen E (2004) Tissue distribution and evolution of fructosamine 3-kinase and fructosamine 3-kinase-related protein. J Biol Chem 279:46606–46613

Drozak J, Veiga-da-Cunha M, Vertommen D, Stroobant V, Van Schaftingen E (2010) Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). J Biol Chem 285:9346–9356

Fortpied J, Gemayel R, Stroobant V, van Schaftingen E (2005) Plant ribulosamine/erythrulosamine 3-kinase, a putative protein-repair enzyme. Biochem J 388:795–802

Fortpied J, Maliekal P, Vertommen D, Van Schaftingen E (2006) Magnesium-dependent phosphatase-1 is a protein-fructosamine-6-phosphatase potentially involved in glycation repair. J Biol Chem 281:18378–18385

Fortpied J, Gemayel R, Vertommen D, Van Schaftingen E (2007) Identification of protein-ribulosamine-5-phosphatase as human low-molecular-mass protein tyrosine phosphatase-A. Biochem J 406:139–145

Galperin MY, Moroz OV, Wilson KS, Murzin AG (2006) House cleaning, a part of good housekeeping. Mol Microbiol 59:5–19

Gemayel R, Fortpied J, Rzem R, Vertommen D, Veiga-da-Cunha M, Van Schaftingen E (2007) Many fructosamine 3-kinase homologues in bacteria are ribulosamine/erythrulosamine 3-kinases potentially involved in protein deglycation. FEBS J 274:4360–4374

Hodge JE (1955) The Amadori rearrangement. Adv Carbohydr Chem 10:169–205

Kim E, Lowenson JD, MacLaren DC, Clarke S, Young SG (1997) Deficiency of a protein-repair enzyme results in the accumulation of altered proteins, retardation of growth, and fatal seizures in mice. Proc Natl Acad Sci USA 94:6132–6137

Krause R, Oehme A, Wolf K, Henle T (2006) A convenient HPLC assay for the determination of fructosamine-3-kinase activity in erythrocytes. Anal Bioanal Chem 386:2019–2025

Leslie RD, Cohen RM (2009) Biologic variability in plasma glucose, hemoglobin A1c and advanced glycation end products associated with diabetes complications. J Diabetes Sci Technol 3:635–643

Mohás M, Kisfali P, Baricza E, Mérei A, Maász A, Cseh J, Mikolás E, Szijártó IA, Melegh B, Wittmann I (2010) A polymorphism within the fructosamine-3-kinase gene is associated with HbA1c Levels and the onset of type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 118:209–212

Monnier VM, Wu X (2003) Enzymatic deglycation with amadoriase enzymes from Aspergillus sp. as a potential strategy against the complications of diabetes and aging. Biochem Soc Trans 31:1349–1353

Moskovitz J (2005) Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. Biochim Biophys Acta 1703:213–219

Pascal SM, Veiga-da-Cunha M, Gilon P, Van Schaftingen E, Jonas JC (2010) Effects of fructosamine-3-kinase deficiency on function and survival of mouse pancreatic islets after prolonged culture in high glucose or ribose concentrations. Am J Physiol Endocrinol Metab 298:E586–E596

Payne LS, Brown PM, Middleditch M, Baker E, Cooper GJ, Loomes KM (2008) Mapping of the ATP-binding domain of human fructosamine 3-kinase-related protein by affinity labelling with 5′-[p-(fluorosulfonyl)benzoyl]adenosine. Biochem J 416:281–288

Peisach E, Selengut JD, Dunaway-Mariano D, Allen KN (2004) X-ray crystal structure of the hypothetical phosphotyrosine phosphatase MDP-1 of the haloacid dehalogenase superfamily. Biochemistry 43:12770–12779

Petersen A, Szwergold BS, Kappler F, Weingarten M, Brown TR (1990) Identification of sorbitol 3-phosphate and fructose 3-phosphate in normal and diabetic human erythrocytes. J Biol Chem 265:17424–17427

Petersen A, Kappler F, Szwergold BS, Brown TR (1992) Fructose metabolism in the human erythrocyte. Phosphorylation to fructose 3-phosphate. Biochem J 284:363–366

Phillips SA, Thornalley PJ (1993) The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. Eur J Biochem 212:101–105

Richard JP (1993) Mechanism for the formation of methylglyoxal from triosephosphates. Biochem Soc Trans 21:549–553

Selengut JD (2001) MDP-1 is a new and distinct member of the haloacid dehalogenase family of aspartate-dependent phosphohydrolases. Biochemistry 40:12704–12711

Selengut JD, Levine RL (2000) MDP-1: a novel eukaryotic magnesium-dependent phosphatase. Biochemistry 39:8315–8324

Szwergold BS (2005) Carnosine and anserine act as effective transglycating agents in decomposition of aldose-derived Schiff bases. Biochem Biophys Res Commun 336:36–41

Szwergold BS (2006) Alpha-thiolamines such as cysteine and cysteamine act as effective transglycating agents due to formation of irreversible thiazolidine derivatives. Med Hypotheses 66:698–707

Szwergold BS, Kappler F, Brown TR (1990) Identification of fructose 3-phosphate in the lens of diabetic rats. Science 247:451–454

Szwergold BS, Howell S, Beisswenger PJ (2001) Human fructosamine-3-kinase: purification, sequencing, substrate specificity, and evidence of activity in vivo. Diabetes 50:2139–2147

Szwergold B, Manevich Y, Payne L, Loomes K (2007) Fructosamine-3-kinase-related-protein phosphorylates glucitolamines on the C-4 hydroxyl: novel substrate specificity of an enigmatic enzyme. Biochem Biophys Res Commun 361:870–875

Van Schaftingen E, Rzem R, Veiga-da-Cunha M (2009) L-2-Hydroxyglutaric aciduria, a disorder of metabolite repair. J Inherit Metab Dis 32:135–142

Veiga-da-Cunha M, Jacquemin P, Delpierre G, Godfraind C, Theate I, Vertommen D, Clotman F, Lemaigre F, Devuyst O, Van Schaftingen E (2006) Increased protein glycation in fructosamine-3-kinase deficient mice. Biochem J 399:257–264

Wiame E, Delpierre G, Collard F, Van Schaftingen E (2002) Identification of a pathway for the utilization of the Amadori product fructoselysine in *Escherichia coli*. J Biol Chem 277:42523–42529

Wiame E, Duquenne A, Delpierre G, Van Schaftingen E (2004) Identification of enzymes acting on alpha-glycated amino acids in *Bacillus subtilis*. FEBS Lett 577:469–472

Wiame E, Lamosa P, Santos H, Van Schaftingen E (2005) Identification of glucoselysine-6-phosphate deglycase, an enzyme involved in the metabolism of the fructation product glucoselysine. Biochem J 392:263–269