Amino Acids (2007) 33: 615–621
DOI 10.1007/s00726-007-0498-z
Printed in The Netherlands

Amino Acids

DNA damage during glycation of lysine by methylglyoxal: assessment of vitamins in preventing damage

G. Suji and **S. Sivakami**

Department of Life Sciences, University of Mumbai, Santacruz (E), Mumbai, India

Received April 8, 2006
Accepted December 6, 2006
Published online February 16, 2007;

Summary. Amino acids react with methylglyoxal to form advanced glycation end products. This reaction is known to produce free radicals. In this study, cleavage to plasmid DNA was induced by the glycation of lysine with methylglyoxal in the presence of iron(III). This system was found to produce superoxide as well as hydroxyl radicals. The abilities of various vitamins to prevent damage to plasmid DNA were evaluated. Pyridoxal-5-phosphate showed maximum protection, while pyridoxamine showed no protection. The protective abilities could be directly correlated to inhibition of production of hydroxyl and superoxide radicals. Pyridoxal-5-phosphate exhibited low radical scavenging ability as evaluated by its TEAC, but showed maximum protection probably by interfering in free radical production. Pyridoxamine did not inhibit free radical production. Thiamine and thiamine pyrophosphate, both showed protective effects albeit to different extents. Tetrahydrofolic acid showed better antioxidant activity than folic acid but was found to damage DNA by itself probably by superoxide generation.

Keywords: Advanced glycation end products – Diabetes – DNA – Free radicals – Glycation – Lysine – Methylglyoxal – Vitamins

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; AGE, advanced glycation end product; MG, methylglyoxal; P, pyridoxine; PLP, pyridoxal-5-phosphate; PM, methylglyoxal, aminoguanidine, pyridoxamine; ROS, reactive oxygen species; T, thiamine; THF, tetrahydrofolic acid; TPP, thiamine pyrophosphate

Introduction

Lysine, an important constituent of proteins, is a part of enzyme active sites and plays critical roles in catalysis. It is also the preferred group for non-enzymatic sugar attachment and subsequent formation of advanced glycation end products (AGEs). Apart from sugars, dicarbonyl compounds formed during intermediate stages of glycation and lipid oxidation can modify the amino groups of lysine resulting in the formation of AGEs and advanced lipoxidation end products, respectively. A wide variety of dicarbonyl compounds are formed endogenously during non-enzymatic glycation and oxidation reactions. Glyoxal, methylglyoxal (MG), 3-deoxyglucosone and malondialdehyde are few such dicarbonyl compounds formed in vivo which have been implicated in the pathophysiology of several diseases.

The three carbon α-dicarbonyl compound, MG, is a by-product of cellular metabolism, formed during spontaneous degradation of triose phosphates, threonine metabolism and autooxidation of sugars (Thornalley, 1993). MG has recently received much attention as a common mediator of AGE formation. Besides elevated tissue concentrations are observed in pathological conditions such as diabetes. MG readily reacts with amino, guanidino and thiol functional groups of proteins to form AGEs causing denaturation of proteins (Lo et al., 1994) and crosslinks like methyl-glyoxal lysine dimer (Wells-Knecht et al., 1996) and N^{ε}-[1-(1-carboxy)ethyl]lysine (Ahmed et al., 1997).

Acute carbonyl stress resulting from elevated levels of dicarbonyls can be genotoxic. Evidence suggests that the AGE product carboxylmethyllysine accumulates in nuclear proteins like histone causing extensive DNA strand cleavage (Roberts et al., 2003). DNA damage was shown to be oxygen dependent and mediated through reactive oxygen species (ROS). In vitro experiments with tubular cells have shown that AGEs and methylglyoxal induce DNA damage, which can be suppressed by antioxidants (Stopper et al., 2004). This finding establishes the involvement of ROS in dicarbonyl mediated DNA damage. Glycation of amino acids with MG was shown to produce ROS. ROS mediated oxidative damage has been hypothe-

sized to play critical roles in diverse biological processes like mutagenesis, carcinogenesis and physiological aging (Sagripanti and Kraemer, 1969).

Efforts to inhibit formation of AGEs by carbonyl-trapping compounds have emerged recently as therapeutic approaches for inhibiting age and disease-dependent changes in biomolecules. Therapeutic interventions have been achieved using both natural as well as pharmaceutical agents. Well-known among these is aminoguanidine (AG), a prototype dicarbonyl scavenging compound. Among other antiglycation molecules, D-lysine, D-penicillamine, diclofenac, desferrioxamine, vitamins and their analogues have been seen to be promising (Rahbar et al., 1999). AGE inhibitors may be dicarbonyl scavengers, metal ion chelators or radical scavengers. Many AGE inhibitors can act by more than one mechanism making it difficult to distinguish whether they owe their antiglycation properties to carbonyl trapping, metal ion chelation or radical scavenging. Supplementation with dietary factors like vitamin B_6 has been shown to reduce diabetic complications (Kannan and Jain, 2004; Suji and Sivakami, 2004). Pyridoxamine, thiamine and thiamine pyrophosphate are carbonyl trapping AGE inhibitors that inhibit the post-Amadori stage in the glycation cascade. Also reports exist wherein the molecules like pyridoxamine have been chemically modified to acquire the desired properties needed for a good inhibitor (Culbertson et al., 2003). However no information exists on the abilities of these vitamins to prevent secondary damage to DNA arising as a consequence of glycation of lysine by MG.

In this study, the late glycation model system consisting of lysine and MG in the presence of Fe^{3+} was used to induce damage to the model DNA pBR322. The abilities of coenzymes pyridoxal-5-phosphate and its precursors pyridoxamine and pyridoxal, thiamine pyrophosphate and its precursor thiamine, tetrahydrofolic acid and its precursor folic acid to prevent oxidative damage to DNA were evaluated.

Materials and methods

Materials

The following chemicals were purchased as indicated: Trolox, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), methylglyoxal, aminoguanidine, pyridoxamine (PM), pyridoxal-5-phosphate (PLP), pyridoxine (P), thiamine (T), thiamine pyrophosphate (TPP), tetrahydrofolic acid (THF) were from Sigma Chemical Co. (St. Louis, MO, USA). Folic acid (FA), 2-deoxy-D-ribose was from Sisco Laboratories (Mumbai, India). DTPA, cytochrome c, thiobarbituric acid, sodium azide were from Spectrochem (Mumbai, India). pBR 322 was from Genei (Bangalore, India). All other chemicals used were of the highest analytical grade.

Analysis of DNA damage

0.5 μg of pBR 322 plasmid DNA in 100 mM potassium phosphate buffer at pH 7.4 was incubated for 2 h at 37 °C with lysine and methyl glyoxal in the presence and absence of Fe^{3+}. The reaction was stopped by freezing. Four microlitres of loading buffer (0.25% bromophenol blue, 40% sucrose) were added and samples analysed by electrophoresis in 0.8% agarose using TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.0) (Kang, 2003). The gels were stained with ethidium bromide and photographed using digital camera (powerShot G2, Canon, Japan). The relative amounts of supercoiled (SC) and open circular (OC) DNA were quantified by the intensities of the band obtained using the image analysis software of Scion Corporation (Frederick, MD, USA). A coefficient of 1.66 was used to correct the lower efficiency of ethidium bromide binding to supercoiled DNA relative to open circular DNA. The percentage of supercoiled DNA, X_{SC}, was calculated by

$$X_{SC} = A_{SC}/(A_{SC} + A_{OC}/1.66) \times 100\%,$$

where A_{SC} and A_{OC} are the intensities for the supercoiled DNA and open circular DNA bands, respectively. Therefore, the percentage of open circular DNA equals $100 - X_{SC}$. The inhibitory activity of vitamins on damage to plasmid was assessed by performing the experiment in the presence of vitamins (Roberts et al., 2003) and inhibition calculated employing the formula:

$$\text{Inhibition}(\%) = \left(1 - \frac{\%\text{OC-DNA (Inhibitor)} - \%\text{OC-DNA (Untreated DNA)}}{\%\text{OC-DNA (Damaged)} - \%\text{OC-DNA (Untreated DNA)}}\right) \times 100$$

With (Damaged) indicating the complete DNA cleavage system in the absence of inhibitor, (Untreated DNA) indicating the plasmid DNA in the absence of any damaging agent and (Inhibitor) indicating the complete cleavage system in the presence of inhibitor.

The trolox equivalent antioxidant capacity (TEAC) assay

$ABTS^{\bullet}$ stock solution was prepared by dissolving 20 mg ABTS in 5 ml of 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet}$ stock solution was diluted with 100 mM phosphate buffer (pH 7.4) to give an absorbance at 734 nm between 0.68 and 0.73. The concentration of $ABTS^{\bullet}$ was determined using a molar extinction coefficient of $1.5 \times 10^4\ M^{-1}\ cm^{-1}$. For measuring the antioxidant capacity, a fixed concentration of the test compounds, 5 μM, was mixed with different concentrations (0–46 μM) of the $ABTS^{\bullet}$ solutions. Absorbance was monitored at 734 nm for 6 min. The total amount of $ABTS^{\bullet}$ scavenged by the antioxidant, i.e. 'a' in the formula $y = a(1 - e^{(-bx)})$ (Sigma Plot) was calculated. In this formula 'y' is the reduction in $ABTS^{\bullet}$ concentration after 6 min and x is the initial $ABTS^{\bullet}$ concentration. The TEAC value was determined using the equation $TEAC = a/(C \times l)$, where C (5 μM) is the concentration of the test compound used in the experiment and l (2.6 μM) is the average concentration of $ABTS^{\bullet}$ that is scavenged per μM of trolox (Rezk et al., 2003).

Measurement of superoxide anion

The generation of superoxide in the aerobic mixture was determined by cytochrome c reduction (Beauchamp and Fridovich, 1971). A reaction mixture contained MG and lysine, 10 μM cytochrome c in 10 mM phosphate buffer (pH 7.4). The reduction rate was determined as the increase in absorbance at 550 nm for 10 min at room temperature.

Measurement of hydroxyl radical

Detection of hydroxyl radicals was carried out by measuring thiobarbituric acid (TBA) reactive 2-deoxy-D-ribose oxidation products (Halliwell and

Gutteridge, 1981). Reaction (200 µl) mixtures containing MG and lysine in the presence or absence of vitamins and 2-deoxy-D-ribose were incubated at 37 °C for 2 h. The degradation of 2-deoxy-D-ribose (100 mM) was then measured by adding 300 µl PBS, 200 µl 2.8% (w/v) trichloroacetic acid, followed by the addition of 400 µl 0.5% (w/v) thiobarbituric acid and heating at 100 °C for 10 min. After cooling, the absorbance was measured at 532 nm.

Results

The results presented in this study show that untreated plasmid DNA showed a major band corresponding to the supercoiled form (I) (Fig. 1A). The incubation of DNA with MG and lysine caused strand breaks (71.7% open circular DNA) as shown by the decrease in the amount of form I and concomitant increase in nicked circular form II. The presence of Fe^{3+} in the system enhanced DNA strand breakage (100% open circular DNA).

Incubation of low concentration of (1 µM) of MG and lysine with 10 µM $FeCl_3$ caused no DNA damage. However 100 µM of MG and lysine in the presence 10 µM $FeCl_3$ resulted in DNA damage (data not shown). Strand breakage was inhibited by azide (74.23%), sodium formate (69.89%) and mannitol (58.9%), known hydroxyl radical scavengers, suggesting the involvement of hydroxyl radical in the damaging process (Fig. 1B). AG, a prototype dicarbonyl scavenger, prevented damage. As reported earlier, AG in the presence of $FeCl_3$ was seen to induce DNA damage (Suji and Sivakami, 2006).

At 5 mM concentration, PLP and P prevented DNA damage but not PM (6.25%). Even at higher concentrations (10, 15, 20 mM) PM showed no effect in preventing damage (Fig. 2A, B). Between P (53.8%) and PLP (100%), the latter was better in its ability to prevent damage. The lowermost concentration of PLP that was effective in the studied system was 1 mM (Fig. 2C). While TPP (61.8%) and T (100%) showed a protective effect at

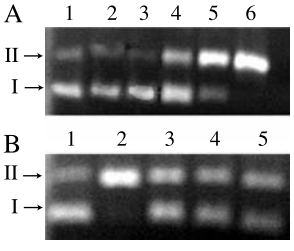

Fig. 1. DNA cleavage during the glycation of lysine by MG. pBR 322 (0.5 µg) was incubated with the following: **A** *1* DNA alone; *2* Lysine (20 mM); *3* MG (20 mM); *4* $FeCl_3$ (100 µM); *5* MG (20 mM) + lysine (20 mM); *6* MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM). **B** *1* DNA alone; *2* DNA + MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM); *3* All the above (lane 2) + azide (250 mM); *4* All the above (lane 2) + Na-formate (250 mM); *5* All the above (lane 2) + mannitol (250 mM)

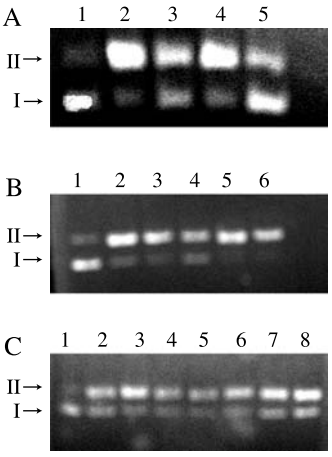

Fig. 2. DNA cleavage during the glycation of lysine by MG in presence of P, PM and PLP. pBR 322 (0.5 µg) was incubated with the following: **A** *1* DNA alone; *2* DNA + MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM); *3* All the above (lane 2) + P (5 mM); *4* All the above (lane 2) + PM (5 mM); *5* All the above + PLP (5 mM). **B** *1* DNA alone; *2* DNA + MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM); *3* All the above (lane 2) + PM (5 mM); *4* All the above (lane 2) + PM (10 mM); *5* All the above (lane 2) + PM (15 mM); *6* All the above (lane 2) + PM (20 mM). **C** *1* DNA alone; *2* DNA + MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM); *3* All the above (lane 2) + P (100 µM); *4* All the above (lane 2) + P (1 mM); *5* All the above (lane 2) + P (2 mM); *6* All the above (lane 2) + PLP (100 µM); *7* All the above (lane 2) + PLP (1 mM); *8* All the above (lane 2) + PLP (2 mM)

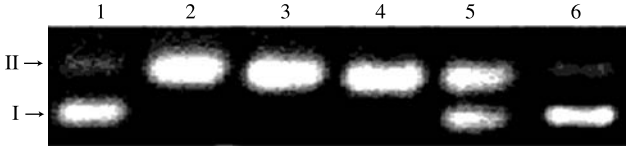

Fig. 3. DNA cleavage during the glycation of lysine by MG in presence of TPP and T. pBR 322 (0.5 µg) was incubated with the following: *1* DNA alone; *2* DNA + MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 µM); *3* All the above (lane 2) + TPP (1 mM); *4* All the above (lane 2) + TPP (5 mM); *5* All the above (lane 2) + TPP (10 mM); *6* All the above (lane 2) + T (10 mM)

10 mM (Fig. 3), FA and THF did not prevent DNA damage at all (Fig. 4A). Besides, THF was found to cause damage when incubated with DNA alone at concentrations as low as 100 µM (Fig. 4B).

Superoxide radical was detected by its ability to reduce ferricytochrome c. A time dependent reduction of cytochrome c was observed. The production of superoxide was inhibited by superoxide dismutase further confirming the formation of superoxide radical (Fig. 5). Table 1 shows the percentage inhibition of cytochrome c reduction by various vitamins. T, PM and FA failed to show inhibition of cytochrome c reduction indicating a lack of ability to scavenge superoxide while TPP, pyridoxine and PLP showed superoxide scavenging abilities. None of the com-

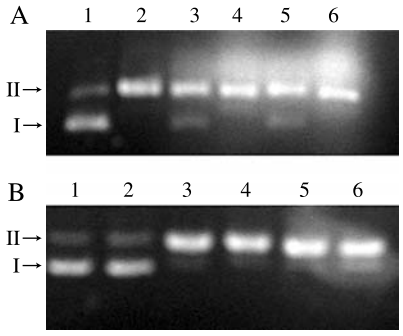

Fig. 4. DNA cleavage during the glycation of lysine by MG in presence of FA and THF. pBR 322 (0.5 μg) was incubated with the following: **A** *1* DNA alone; *2* MG (20 mM) + lysine (20 mM) + $FeCl_3$ (100 μM); *3* All the above (lane 2) + FA (5 mM); *4* All the above (lane 2) + THF(5 mM); *5* All the above (lane 2) + FA (10 mM); *6* All the above (lane 2) + THF (10 mM). **B** *1* DNA alone; *2* $FeCl_3$ (50 μM); *3* THF (100 μM); *4* THF (100 μM) + $FeCl_3$ (50 μM); *5* THF (1 mM); *6* THF (1 mM) + $FeCl_3$ (50 μM)

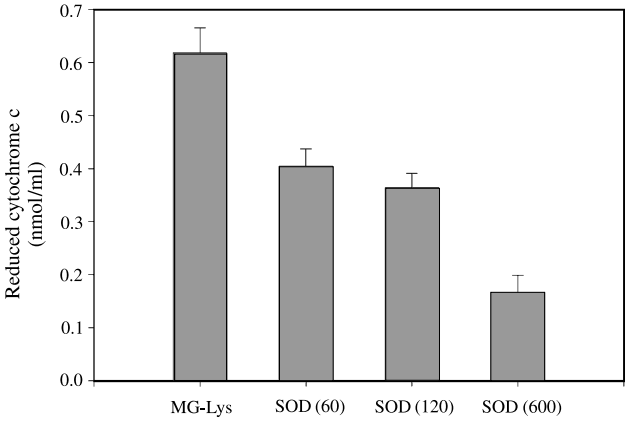

Fig. 5. Superoxide formation during the glycation of lysine by MG. MG (10 mM) and lysine (10 mM) was reacted in presence of various concentrations of superoxide dismutase. 60 units SOD (60), 120 units SOD (120), 600 units SOD (600)

Table 1. Inhibition (%) of superoxide generation by vitamins in ferricytochrome c reduction assay system

Vitamins (mM)	0.5	1.0	2.0	5.0	10.0
Thiamine	0.0	0.0	0.0	0.0	0.0
Thiamine pyrophosphate	22.4	22.4	22.4	31.5	59.89
Pyridoxine	15.6	31.9	31.9	35.8	45.27
Pyridoxamine	0.0	0.0	0.0	0.0	0.0
Pyridoxal-5-phosphate	35.2	44.9	51.4	54.6	70.8

pounds tested except THF directly reduced cytochrome c in blank reactions.

Hydroxyl radical production expressed as TBARS nmoles/ml was found to increase as a function of MG and lysine. Addition of metal ions further increased the production of TBARS. At a constant lysine concentration

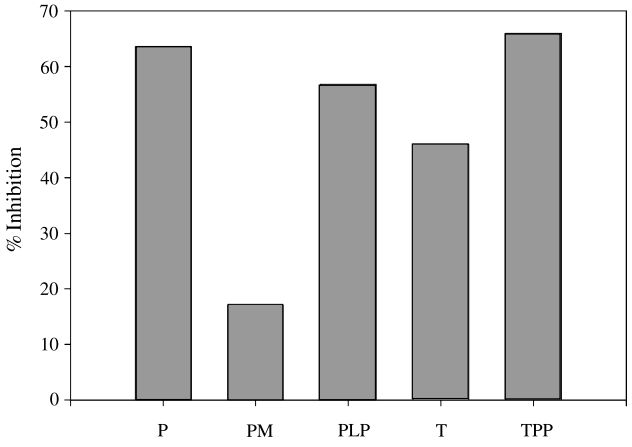

Fig. 6. Percentage inhibition of hydroxyl radical production by vitamins in MG and lysine reaction system. MG and lysine (50 mM) each were incubated in the presence of vitamins (50 mM) and deoxy ribise for 2 h. The degradation of 2-deoxy-D-ribose was then determined as described in Materials and methods

of 50 mM, hydroxyl radical production increased with increasing MG. In the presence of Fe^{3+}(100 μM), the hydroxyl radical production was higher. Similarly the effect of increasing lysine concentration at a constant MG concentration of 50 mM, showed increased hydroxyl radical production in the presence of Fe^{3+} (100 μM). At concentrations of lysine below 10 mM and at fixed concentration of 50 mM MG, Fe^{3+} had no effect on TBARS production (data not shown).

Though all vitamins tested showed abilities to prevent hydroxyl radical production to different extents, TPP and P showed maximum abilities followed by PLP, T and PM (Fig. 6). PLP and P showed marginal differences in their abilities to prevent hydroxyl radical production in this system. No conclusive results could be obtained with FA and THF as they precipitated at the low pH of the assay.

The free radical scavenging of vitamins were evaluated by means of ABTS assay, wherein the TEAC values give

Table 2. TEAC of vitamins (for details see Materials and methods)

Vitamins	a	l × C	TEAC
Pyridoxal	19.69 ± 1.01	13.25	1.48 ± 0.14
Pyridoxamine	21.86 ± 0.58	13.25	1.64 ± 0.03
Pyridoxal-5-phosphate	3.40 ± 0.05	13.25	0.25 ± 0.07
Thiamine	4.24 ± 0.16	13.25	1.07 ± 0.01
Thiamine pyrophosphate	2.46 ± 0.12	13.25	0.18 ± 0.01
Folic acid	7.74 ± 0.27	13.25	0.58 ± 0.02
Tetrahydro folic acid	19.03 ± 0.69	13.25	1.43 ± 0.07

C Concentration of compound = 5 μM
l ABTS consumed by 1 μM of trolox = 2.65 μM
a Total ABTS scavenged by the compound

an indication of the radical scavenging ability of the molecule. As seen from Table 2, differences in antioxidant activities were observed between vitamins. Based on the TEAC, it can be observed that the number of radicals that can be scavenged by the vitamins varies. PM was highly efficient (TEAC 1.64 ± 0.03), followed by pyridoxine (TEAC 1.48 ± 0.141). However PLP showed a low antioxidant activity (TEAC 0.25 ± 0.07). Among the vitamins used, TPP showed the lowest TEAC value (TEAC 0.18 ± 0.01). By comparison thiamine, which lacks the pyrophosphate group, showed better antioxidant activity (TEAC 1.07 ± 0.01). Compared to FA (TEAC 0.58 ± 0.028) the reduced form THF possessed better antioxidant activity (TEAC 1.43 ± 0.07).

Discussion

The model system of methylglyoxal and amino acids has been used to study the late stages of glycation between dicarbonyl intermediates formed during glycation and free amino groups of proteins (Yim et al., 1995). The reaction of methylglyoxal with amino acids generates cross-linked methylglyoxal dialkylimine radical cation (Fig. 7, structure a), the enediol radical anion of methylglyoxal (Fig. 7, structure b), and the superoxide radical anion (Fig. 7, structure c). A direct 1-electron transfer between a Schiff base methylglyoxal dialkylimine (or its protonated form) and methylglyoxal is responsible for the generation of the cross-linked radical cation and the radical counteranion of methylglyoxal (Fig. 7, *step iii*). Under aerobic conditions, molecular oxygen can then accept an electron from the methylglyoxal anion to generate the superoxide radical anion (Fig. 7, *step iv*). The formation of methylglyoxal dialkylimine radical cation and the enediol radical anion of methylglyoxal were independent of molecular oxygen or metal ions. The later stage needs the presence of molecular oxygen. The formation of α-ketoaldehydes during glycation is a critical step that leads to protein cross-linking and formation of radical cation sites on the cross-linked proteins. The counteranions, superoxide and hydrogen peroxide generated from MG anion can initiate free radical chain reactions leading to damage of biomacromolecules in close proximity to reaction sites.

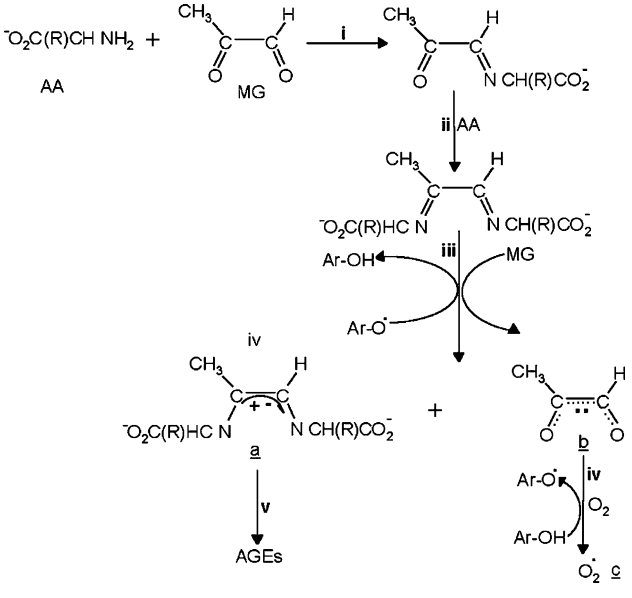

Fig. 7. Reaction scheme for the reaction of methylglyoxal (MG) with amino acids (AA) and probable site of action of phenolic antioxidants (Ar-OH)

The pBR 322 is a sensitive indicator of single strand breaks arising due to damage by glycation and free radicals (Kang, 2003; Levi and Werman, 2001; Mullokandov et al., 1994; Pischetsrieder et al., 1999). In this study the damage to pBR 322 was caused by late stage glycation model system comprising of MG and lysine. The damage to DNA was found to be mediated by free radicals. High concentrations of MG used in this study may not be physiologically attainable, but were essential to induce visible structural damage that could be quantitated. However we did observe damage at concentration as low as 100 μM of MG and lysine. A similar study showed that even 1 μM of MG and lysine could induce damage (Kang, 2003).

Radical trapping antioxidants have been known to inhibit the glycation reaction (Khalifah et al., 1999; Quattrucci and Masci, 1992; Vinson and Howard, 1996). This study revealed that there was a strong correlation between inhibition of production of superoxide and hydroxyl radical and protection of DNA. This establishes that in the MG lysine system, damage was caused by intermediate radical species formed during glycation. PLP and P were able to protect PBR 322 and showed superoxide and hydroxyl radical scavenging. PM lacked DNA protection ability and also showed low hydroxyl and no superoxide scavenging ability. PM has been shown to have only weak hydrogen donating (radical-trapping) ability making it a marginally effective antioxidant. The electron donating para substituents have a strong influence on the radical trapping ability of PM, as they lower the phenolic O–H bonding dissociation enthalpies (Delange and Glazer, 1989; Onorato et al., 2000). Though PM can form Schiff-base with the carbonyl of MG, its inability to protect DNA against damage may be due to the more rapid reaction of amino group of lysine with MG and consequent

nonavailability of free carbonyl groups for reaction with PM. In contrast though PLP had the lowest TEAC value, it was seen to reduce superoxide and hydroxyl radical generation while PM and P which showed higher TEAC values were poorer free radical scavengers. It has therefore been suggested that the ability of PLP to inhibit AGE formation may be due to internal hemiacetal formation (Booth et al., 1996). This may be relevant to the Schiff-base condensation of PLP with the amino group of lysine, which may have better antioxidant and free radical scavenging potentials.

Similarly TPP showed lower antioxidant activity than free thiamine, the only difference between them being the presence of a pyrophosphate group on TPP. Since both thiamine and its phosphorylated forms have amine functional group, the marked difference in the antioxidant between them may be attributed to the pyrophosphate. Our finding that thiamine had no superoxide scavenging ability is supported by earlier work using pyrogallol as well as xanthine oxidase-hypoxanthine assays (Hu et al., 1995). It is possible that radical scavenging phenolic antioxidants reduce radical intermediates such as enediol anions and cross-linked methylglyoxal dialkylimine cations and convert them to their parent non radical forms (Culbertson et al., 2003).

Folates function as cofactors in the transfer of single carbon moiety in metabolic reactions. A difference in TEAC seen in case of FA and THF can be explained on the basis of substituents on the amino pyrimidine ring. It has been shown that the 4-hydroxy-2,5,6-triaminopyrimidine is of major importance in the antioxidant activity of folates (Rezk et al., 2003). The location of a potent electron donating group in the reduced form at the ortho position to the 4-hydroxyl group may explain the potent antioxidant activity of THF as compared to FA. The DNA protecting ability of FA was difficult to interpret in view of the intrinsic fluorescence of folates. However THF alone induced damage to DNA, probably due to autoxidation and formation of free radicals. This was also confirmed by observation that THF could reduce cytochrome c.

From the above results it can be concluded that vitamins behave differently in their abilities to protect DNA against glycation mediated damage. Vitamin B6 has been shown to be beneficial to diabetes and neurodegenerative diseases in varying degrees. The three water soluble forms of vitamin B_6 pyridoxine, pyridoxal phosphate and pyridoxamine are interconvertible. Our findings gain importance in view of the higher incidence of mutation and DNA damage, elevated concentrations of MG in blood (McLellan et al., 1994) and pyridoxine deficiency in both type I and II diabetics (Kannan and Jain, 2004).

References

Ahmed MU, Frye EB, Degenhardt TP, Thorpe SR, Baynes JW (1997) N^{ε}-[1-(1-carboxy)ethyl]lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J 324: 565–570

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276–287

Booth AA, Khalifah RG, Hudson BG (1996) Thiamine pyrophosphate and pyridoxamine inhibit the formation of antigenic advanced glycation end-products: comparison with aminoguanidine. Biochem Biophys Res Commun 220: 113–119

Culbertson SM, Vassilenko EI, Morrison LD, Ingold KU (2003) Paradoxical impact of antioxidants on post-Amadori glycoxidation. J Biol Chem 278: 38384–38394

Delange RJ, Glazer AN (1989) Phycoerythrin fluorescence-based assay for peroxy radicals: a screen for biologically relevant protective agents. Anal Biochem 177: 300–306

Halliwell B, Gutteridge JM (1981) Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS Lett 128: 347–352

Hu ML, Chen YK, Lin YF (1995) The antioxidant and prooxidant activity of some B vitamins and vitamin-like compounds. Chem Biol Interact 97: 63–73

Kang JH (2003) Oxidative damage of DNA by the reaction of amino acid with methylglyoxal in the presence of Fe(III). Int J Biol Macromol 33: 43–48

Kannan K, Jain SK (2004) Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H_2O_2-treated U937 monocytes. Free Radic Biol Med 36: 423–428

Khalifah RG, Baynes JW, Hudson BJ (1999) Amadorins: novel post-Amadori inhibitors of advanced glycation reactions. Biochem Biophys Res Commun 257: 251–258

Levi B, Werman MJ (2001) Fructose triggers DNA modification and damage in Escherichia coli plasmid. J Nutr Biochem 12: 235–241

Lo TW, Westwood ME, Mclellan AC, Selwood T, Thornalley PJ (1994) Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. J Biol Chem 269: 32299–32305

McLellan AC, Thornalley PJ, Benn J, Sonksen PH (1994) The glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci 87: 21–29

Mullokandov EA, Franklin WA, Brownlee M (1994) DNA damage by the glycation products of glyceraldehydes 3-phosphate and lysine. Diabetologia 37: 145–149

Onorato JM, Jenkins AJ, Thorpe SR, Baynes JW (2000) Pyridoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. J Biol Chem 275: 21177–21184

Pischetsrieder M, Siedel W, Munch G, Schinzel R (1999) N(2)-(1-carboxyethyl)deoxy guanosine, a non enzymatic glycation adduct of DNA, induces single strand breaks and increases mutation frequencies. Biochem Biophys Res Commun 264: 544–549

Rahbar S, Yernini KK, Scott S, Gonzales N, Lalezar I (1999) Novel inhibitors of advanced glycation end products. Biochem Biophys Res Commun 262: 651–656

Rezk BM, Haenen GRMM, van der Vijagh WJF, Bast A (2003) Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. FEBS Lett 555: 601–605

Roberts MJ, Wondrak GT, Laurean DC, Jacobson Mk, Jacobson EL (2003) DNA damage by carbonyl stress in human skin cells. Mutat Res 260: 115–119

Sagripanti J-L, Kraemer KH (1969) Site-specific oxidative DNA damage at polyguanosines produced by copper plus hydrogen peroxide. J Biol Chem 264: 1729–1734

Stopper H, Schupp N, Bahner U, Sebekova K, Klassen A, Heidland A (2004) Genomic damage in end-stage renal failure: potential involvement of advanced glycation end products and carbonyl stress. Semin Nephrol 24: 474–478

Suji G, Sivakami S (2004) Glucose, glycation and aging. Biogerontology 5: 365–373

Suji G, Sivakami S (2006) DNA damage by free radical production by aminoguanidine. Ann NY Acad Sci 1067: 191–199

Thornalley PJ (1993) The glyoxalase system in health and diseases. Mol Aspects Med 14: 287–371

Vinson JA, Howard TB (1996) Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. J Nutr Biochem 7: 659–663

Well-Knecht KJ, Brinkmann E, Well-Knecht MC, Litchfield JE, Ahmed MU, Reddy S, Zayak DV, Thorpe SR, Baynes JW (1996) New biomarkers of Maillard reaction damage to proteins. Nephrol Dial Transplant 11: 41–47

Yim H-S, Kang S-O, Hah Y-C, Chock PB, Yim MB (1995) Free radicals generated during the glycation reaction of amino acids by methyl glyoxal. J Biol Chem 270: 28228–28233

Authors' address: S. Sivakami, Department of Life Sciences, University of Mumbai, Santacruz (E), Mumbai 400 098, India,
E-mail: sivakami_s2000@yahoo.com