Research paper

Autoimmune response to AGE modified human DNA: Implications in type 1 diabetes mellitus☆

Saheem Ahmad [a,b], Moin Uddin [a,*], Safia Habib [d], Uzma Shahab [a,c], Khursheed Alam [a], Asif Ali [a]

[a] Department of Biochemistry, J.N. Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, India
[b] Department of Biosciences/Biochemistry, Integral University, Lucknow, UP, India
[c] Department of Biochemistry, Central Drug Research Institute, Lucknow, UP, India
[d] Biochemistry Section, Women's College, Aligarh Muslim University, Aligarh, India

ARTICLE INFO

ABSTRACT

Aims: Non-enzymatic glycation of DNA both *in vivo* and *in vitro* results in generation of free radicals, known as glycoxidation. Glycoxidation leads to structural perturbation of DNA resulting in generation of neo-antigenic epitopes having implication in autoimmune disorders like diabetes mellitus. In this study human placental DNA was glycated with methylglyoxal (MG) and lysine (Lys) in the presence of Cu^{2+} and its auto-antibody binding was probed in Type 1 diabetes patients.
Methods: Glycation was carried out by incubating DNA with MG, Lys and Cu^{2+} for 24 h at 37 °C. Carboxyethyl deoxyguanosine (CEdG) formed in glycation reaction was studied by LC-MS and the pathway for Amadori formation was studied by ESI-MS techniques. Furthermore, binding characteristics of auto-antibodies in diabetes patients were assessed by direct binding, competitive ELISA and band shift assay.
Results: DNA glycation with MG, Lys and Cu^{2+} results in the formation of CEdG (marker of DNA glycation) which was confirmed by LC-MS. The intermediate stages of glycation were confirmed by ESI-MS technique. Serum from diabetes patients exhibited enhanced binding and specificity for glycated DNA as compared to native form.
Conclusions: Glycation of DNA has resulted in structural perturbation causing generation of neo-antigenic epitopes thus recognizing auto-antibodies in diabetes.

Introduction

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia due to the deficiency of insulin or insulin resistance [1]. Hyperglycemia has an important role in the pathogenesis of diabetes complications by increasing glycation intermediates and the gradual build-up of advanced glycation end-products (AGEs) in body tissues [1,2]. Glycoxidation and AGEs are accompanied by increased free radical activity that contributes toward the biomolecular damage in diabetes. There is a considerable interest in receptors for AGEs (RAGE) found on many cell types, particularly those affected in diabetes [3]. Recent studies suggests that interaction of AGEs with RAGE alter intracellular signaling, gene expression, release of pro-inflammatory molecules and free radicals that contribute toward the pathology of secondary complications [4]. Hyperglycemia has an important role in the pathogenesis of long-term complications and diabetes patients with poor blood glucose control are particularly at risk [4]. Furthermore, complications appear to affect organs where cells do not require insulin for glucose uptake, such as those of the nervous system, heart, kidneys and small blood vessels [1]. As a consequence, these cells have high concentrations of intracellular glucose during hyperglycemia. The precise role of hyperglycemia in the pathogenesis of long-term complications is still unclear. However, it is very well reported that under hyperglycemic condition the level of methylglyoxal (MG) increases 5–6 fold in the patients suffering from diabetes [5]. Moreover, the concentration of MG in human lenses is normally about 20 times higher than in plasma [6]. It has been shown that physiological levels of MG can induce DNA cleavage and ROS generation and decrease cellular adhesion in mononuclear cells [7]. Thus, MG has direct role in the initiation and progression of the glycation reaction.

Glycation is the nonenzymatic addition of reducing sugars as well as compounds related to sugars such as ascorbic acid, MG, glyoxal and 3-deoxyglucosone etc. into biological macromolecules such as DNA [8]. The free carbonyl groups of the sugar and related moieties react with the free amino residues of the macromolecules in a series of chemical processes known as Maillard reaction. Initiation of glycation occurs by the formation of acid-labile Schiff base adducts which undergoes Amadori rearrangements into more stable products [9]. The early glycation products undergo slow transformation to yield the irreversible AGEs. These reactions have recently attracted significant attention because of their association with free radicals, which play roles in the development of cancer, diabetes, heart disease, cataract, atherosclerosis and neurodegenerative disorder. Previous investigations by several scientists using biochemical and molecular biological methods have shown that DNA structure and function are affected by the addition of sugars, resulting in deleterious modifications and mutations [10–12].

In the present study commercially available human placental DNA was glycated as described previously [13]. The structural changes induced in the DNA macromolecule by MG and Lys in the presence of Cu2+ have been studied extensively. The adduct formed by MG-Lys-Cu^{2+} system to human DNA was probed by LC-MS technique. Moreover, the ESI-MS technique is also employed to see the glycation intermediates formed in the DNA glycation. Furthermore, the glycated DNA was used as an antigen for detecting anti-DNA antibodies in sera of type 1 diabetes patients by direct binding, competitive inhibition ELISA and the band shift assay.

Materials and methods

Materials

Methylglyoxal (MG), anti-human IgG alkaline phosphatase conjugates, p-nitrophenyl phosphate, sodium dodecyl sulfate, Tween-20, Protein A-agarose (2.5 ml pre-pack column) and dialysis tubing were purchased from Sigma Chemical Company, U.S.A. Dihydroxy acetone (DHA) was from Merck, Germany. Lysine was from Sisco research laboratory. Triton X-100 was procured from Hi−Media. Trizma base was from Spectrochem, Mumbai, India. ELISA plates (96 wells) were purchased from NUNC, Denmark. Acrylamide, bisacrylamide, ammonium persulphate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were from Bio-Rad Laboratories, U.S.A. EDTA, (disodium salt), silver nitrate, sodium carbonate and sodium nitrite were from Qualigens, India. All other reagents/chemicals were of the highest analytical grade available.

Collection of sera

Fasting blood samples of type 1 diabetes patients, were obtained from J.N. Medical College Hospital, A.M.U, Aligarh after the informed consent. None of the patients with diabetes had other autoimmune diseases. Specifically, there were no cases of lupus or other connective tissue disorders. Normal human sera were obtained from healthy subjects. Samples were collected in a glass test tube and left to clot for 30 min at 37 °C. Serum was separated by centrifugation at 3000 rpm for 10 min. Serum samples were then heated at 56 °C for 30 min to inactivate complement proteins and stored in aliquots at −20 °C with 0.1% sodium azide as preservative.

Purification of human placental DNA

Commercially available human placental DNA was purified free of proteins and single stranded regions as described previously [14].

Modification of human placental DNA by methylglyoxal (MG) and lysine in presence of Cu^{2+}

Human placental DNA was modified by MG and lysine in the presence and absence of Cu^{2+} as described by Ahmad et al. (2011) [13]. 37.8 μM of human DNA was thoroughly mixed with MG (40 mM), lysine (40 mM) and Cu^{2+} (300 μM) in 10 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl and incubated at 37 °C for 24 h followed by extensive dialysis against PBS to remove unbound constituents.

Synthesis of the standard, carboxyethyl deoxyguanosine (CEdG)

The synthesis of CEdG was carried out as described by Ashraf et al. (2012) with slight modifications [15]. Briefly, 50 mg of deoxyguanosine suspended in 1 ml of 100 mM phosphate buffer (pH, 7.4), was incubated with 100 mg of dihydroxyacetone at 70 °C in a shaking water bath. It got dissolved at 70 °C in the course of reaction after 24 h. CEdG was isolated by preparative HPLC using 50 mM ammonium acetate buffer solution and methanol as eluents.

Detection of a glycated product, CEdG in modified human placental DNA by LC-MS

An Agilent 1100 capillary HPLC System equipped with a Synergi C_{18} analytical column was used for HPLC analysis of native and modified analog of human placental DNA. General chromatographic conditions were as follows: C_{18} column (2 mm × 150 mm with 4 μm particle size); eluant A, 5 mM aqueous ammonium acetate buffer, pH 7; eluant B, Acetonitrile (CH_3N) gradient solution the CH_3N concentration was raised from 0 to 4.0% in the first 5 min; from 4.0 to 6.5% over 30 min; held at 6.5% for 5 min, and then raised to 90% to wash residual material off the column at a constant flow rate of 500 μL/min. DNA bases were detected by diode array detector (DAD) at 254 nm, their absorption maximum. LC-MS analyzes of CEdG standard were carried out using a Micromass Quattro Ultima Triple Quadrupole Mass Spectrometer interfaced to an Agilent 1100 capillary HPLC system.

Characterization of DNA-AGEs by electrospray ionization mass spectrometry

An orthogonal time of flight (TOF) mass spectrometer (Applied Biosystems Mariner Atmospheric Pressure Ionization TOF Workstation, Framingham, MA, USA) equipped with standard electrospray ionization source was used. The mass spectral data were collected at positive ion polarity. Nitrogen was used as the nebulizer, heater and collision gas. The sciex heater was set to 350 °C and the spray tip potential was set at 4000 V. The instrument was outfitted with an integrated syringe pump with a dual syringe rack for direct infusion onto the mass spectrometer. The mass spectrometry system was operated on full scan mode (m/z 100−1000). Spectral acquisition was performed every 2 s and a total of ten spectra were accumulated. The final spectrum depicts an average of 4−6 scans.

Enzyme linked immuno-sorbent assay (ELISA)

ELISA was carried out on flat bottom polystyrene plates as described earlier [16,17]. Briefly, microtitre wells were coated with one hundred microliter of 2.5 μg/ml of DNA (in TBS, pH 7.4) and incubated for 2 h at 37 °C and overnight at 4 °C. Each sample was coated in duplicate and half of the plate, devoid of antigen, served as control. The test-plate wells were emptied and washed thrice

with TBS-T to remove the unbound antigen. Unoccupied sites were blocked with 150 µl of 1.5% non-fat dry milk (in TBS, pH 7.4) for 4–5 h at 4 °C followed by single wash with TBS-T. In direct binding ELISA, antibodies were directly added into antigen-coated wells and incubated for 2 h at 37 °C and overnight at 4 °C respectively. The wells were emptied and extensively washed with TBS-T. Anti-immunoglobulin G alkaline phosphatase conjugate was added to each well and incubated at 37 °C for 2 h and then the plates were washed thrice with TBS-T followed by a single wash with distilled water. Para-nitrophenyl phosphate was added and the developed color was read at 410 nm on a microplate reader. The results were expressed as mean of difference of absorbance values in test and control wells ($A_{test} - A_{control}$).

Competition ELISA

The specific binding characteristics of antibodies were ascertained in competitive binding assay [18]. Varying amounts of inhibitors (0–20 µg/ml) were mixed with constant amount of antiserum or IgG. The mixture was incubated at room temperature for 2 h and overnight at 4 °C. Immune complex thus formed was coated in the wells instead of the serum. The remaining steps were the same as in direct binding ELISA.

Percent inhibition was calculated using the formula:

$$Percent\ inhibition\ =\ 1 - \left(\frac{A_{inhibited}}{A_{uninhibited}}\right) \times 100$$

Gel retardation assay

Antigen-antibody specificity was further confirmed by the gel retardation assay. A constant amount of DNA antigen (0.5 µg) was mixed with varying amounts of IgG [19] and incubated for 2 h at 37 °C and overnight at 4 °C. At the end of incubation, one-tenth volume of sample buffer was added to antigen–antibody complex and electrophoresed on 1% agarose gel in TAE buffer (pH 7.8) for 2 h at 30 mA current. The gels were stained with ethidium bromide (0.5 mg/ml) and visualized under UV light and photographed.

Statistics

Data are presented as mean ± SD. Statistical significance of the data was determined by student's t-test (Statgraphics, Origin 6.1). A value of $p < 0.05$ was considered statistically significant.

Results

Glycation of human DNA

Human DNA (37.8 µM) was glycated with 40 mM MG, 40 mM lysine and 150 µM Cu^{2+} for 24 h at 37 °C. The structural perturbation caused to the structure of the DNA macromolecule as a consequence of the glycation reaction has been confirmed in the previously published literature [13,14].

Synthesis and characterization of N^2-(1-carboxyethyl)-2-deoxyguanosine (CEdG)

Synthesis of the standard, CEdG was performed as described previously [15]. After final preparation, CEdG was isolated by preparative HPLC using 50 mM ammonium acetate buffer solution and methanol as eluents. The elution of CEdG was obtained at a retention time of 14.399 min when UV detector was used for the experiment. However, deoxyguanosine (dG) gave elution at a retention time of 9.1 min (data not shown).
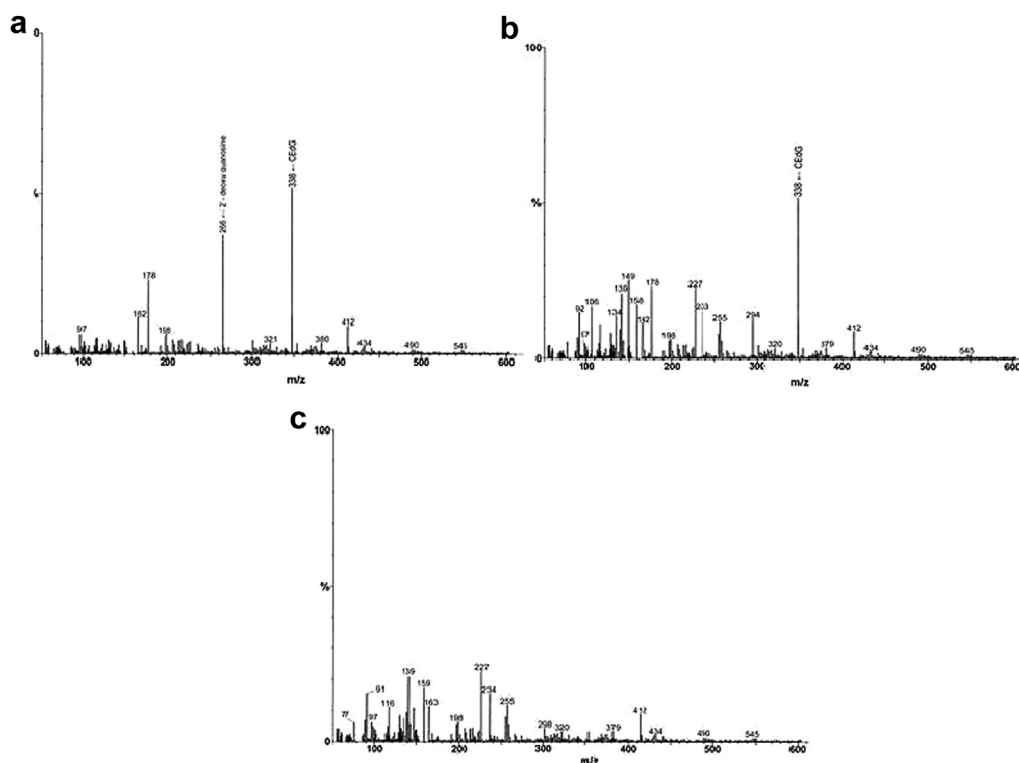
Figure 1. a) Full scan LC-MS spectral analysis of synthesized CEdG standard. b) Full scan LC-MS spectral analysis of hydrolyzed modified human DNA (1 mg/ml). c) Full scan LC-MS spectral analysis of hydrolyzed native human DNA (1 mg/ml).
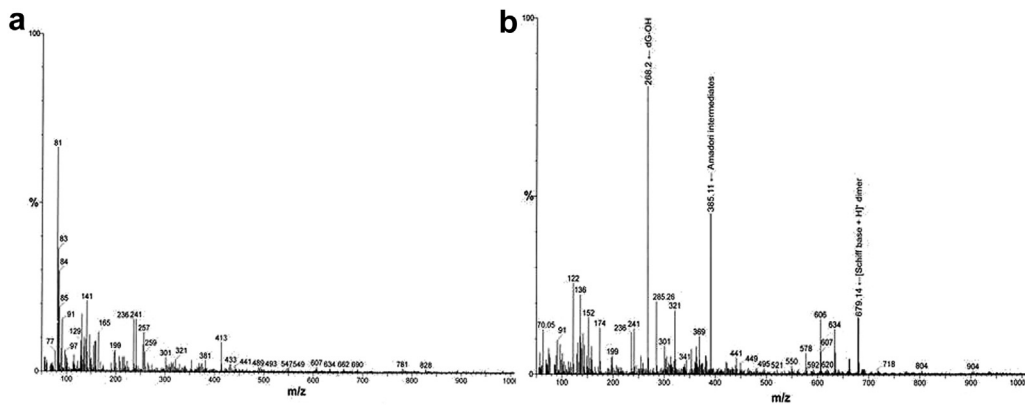
Figure 2. a) Full scan ESI-MS spectral analysis of native human DNA (1 mg/ml) of HPLC resolved products. b) Full scan ESI-MS spectral analysis of modified human DNA (1 mg/ml) of HPLC resolved glycated products.

Detection of N^2-(1-carboxyethyl)-2-deoxyguanosine (CEdG) formed in modified human DNA by LC-MS

The CEdG synthesized from dG was analyzed on mass spectrometer which showed a mass (m/z) of 338 significantly different from the 266 mass of dG (Fig. 1a). When modified DNA was analyzed under identical conditions they showed mass value matching with CEdG [15] (Fig. 1b). The MG-Lys-Cu^{2+} glycated-DNA suggests CEdG formation and m/z value of 338 was seen in modified human DNA [15] (Fig. 1b). However, analysis of native DNA suggests no CEdG formation as evidence from figure (Fig. 1c).

Characterization of DNA-AGEs by electrospray ionization mass spectrometry (ESI-MS)

In an attempt to confirm the formation of Schiff base and Amadori product in glycated DNA, mass spectrometry was used to analyze the hydrolyzed glycated human DNA. Fig. 2 a&b show the respective mass–spectral profiles of hydrolyzed native and MG-Lys-Cu^{2+} glycated human DNA. The ion at m/z 341 is consistent with a [Schiff base + H]$^+$ molecule resulting from the condensation reaction of dG (Mr 285.26) with methylglyoxal (Mr 70.06) in a dehydration reaction involving the loss of a water molecule. The ion at m/z 679 is consistent with the formation of a [Schiff base + H]$^+$ dimer product. Moreover, the ion at 268 is speculated to result from the loss of a hydroxyl group from dG. Furthermore, the ion with m/z 385 is assumed to be fragment formed by the degradation of MG reacting with Schiff base product, or its enaminol or Amadori intermediates.

Immunogenicity of modified DNA

The glycated DNA has been found to be a potent immunogen inducing high titer (>1:12800), highly specific and non-precipitating antibodies in experimental animals [13].

Binding of autoantibodies against native and MG-Lys-Cu^{2+} modified human DNA in diabetes patients

The pilot study was performed to screen out the positive sera samples (sera showing higher binding with the immunogen) from type 1 diabetes patients. The sera were obtained from patients attending J.N. Medical College and Hospital, A.M.U., Aligarh after the informed consent. Our study comprised of 40 serum samples of type 1 diabetes mellitus patients. Control serum samples from age and sex matched individuals were obtained from 20 normal healthy subjects. All sera were diluted to 1:100 in TBS-T and subjected to direct binding ELISA on solid phase separately coated with equal amounts of native and MG-Lys-Cu^{2+} modified human DNA. Out of 40 sera from type 1 diabetes, 27 samples (67.5%) showed higher binding with the glycated DNA as compared to the native form (Fig. 3). The serum samples which showed enhanced binding (double or more than double binding) were considered for further studies, while samples whose absorbance was less than or equal to control were not included.

Immuno-cross reactivity of autoantibodies from diabetes type 1 patients

Competition ELISA was carried out to analyze the specific binding of circulating autoantibodies in Type 1 diabetes patients sera for native and MG-Lys-Cu^{2+} modified human DNA. In the 27 sera chosen from Type 1 diabetes patients, which showed enhanced binding, the observed maximum inhibition with MG-Lys-Cu^{2+} modified human DNA was in the range of 46.9–63.1% while with native human DNA it ranged from 20.2 to 33%. Mean inhibition for the entire sample tested with native human DNA was 26.98 ± 3.8%, while for MG-Lys-Cu^{2+} modified human DNA, it was 54.95 ± 5.4%. However, under similar experimental conditions normal human subjects showed mean inhibition of 32.5 ± 2.1% with MG-Lys-Cu2+
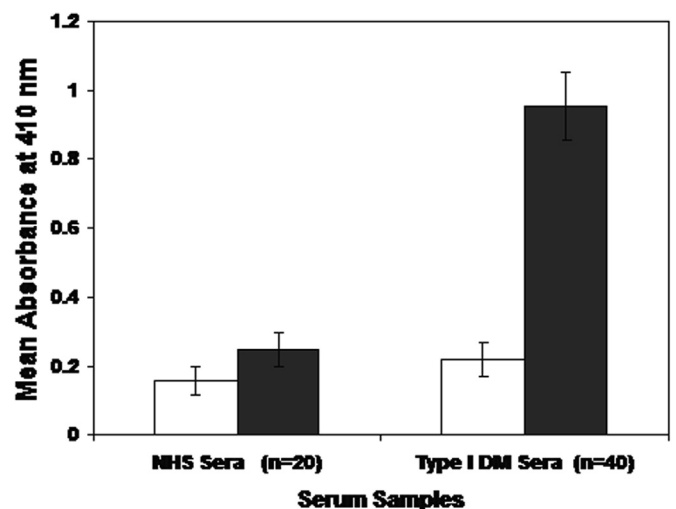
Figure 3. Direct binding ELISA of serum antibodies from diabetes mellitus (DM) patients to native human DNA (□) and MG-Lys-Cu^{2+} glycated human DNA (■). Serum from normal human subjects (NHS) served as control. The microtitre plates were coated with the MG-Lys-Cu^{2+} glycated human DNA (2.5 μg/ml). $p < 0.001$ vs native human DNA.

Table 1a
Competitive inhibition data of serum auto-antibodies in type 1 diabetes patients

| Sera no | Disease duration (years) | Maximum percent inhibition at 20 µg/ml | |
		Native human DNA DNA	MG-Lys-Cu^{2+} modified human
01	6	26.8	51.0
03	8	22.9	54.0
04	7	29.6	49.0
07	8	24.5	52.8
08	10	28.8	58.0
09	6	31.0	46.9
10	7	24.0	50.0
11	8	27.4	53.0
14	18	30.7	61.0
15	14	26.8	52.5
17	18	28.0	62.0
18	16	30.0	58.0
20	12	29.0	54.0
21	17	26.0	62.0
22	6.5	20.8	48.0
23	8	22.0	50.0
24	18	24.8	63.1
25	16	28.0	57.0
28	15	29.0	47.0
29	15	31.0	48.0
31	15	33.0	61.3
32	>20	26.0	62.0
34	15	27.8	55.7
36	10	24.0	49.0
37	16	24.7	62.8
38	15	20.2	58.6
39	15	31.7	52.8
Mean ± SD		26.98 ± 3.8%	54.8 ± 5.4%
NHS		24.4 ± 2.5%	32.5 ± 2.1%

The microtitre plates were coated with MG-Lys-Cu^{2+} modified human DNA (2.5 µg/ml).
NHS: Normal Human Sera.

Table 1b
Competitive inhibition data of IgG isolated from type 1 diabetes patients

| Sera no | Disease duration (years) | Maximum percent inhibition at 20 µg/ml | |
		Native human DNA DNA	MG-Lys-Cu^{2+} modified humans
03	8	28.0	62.1
07	8	29.6	60.7
08	10	31.7	65.1
14	18	33.8	68.0
15	14	30.1	61.9
17	18	28.0	69.0
18	16	33.8	64.1
21	17	35.6	65.8
24	18	31.0	68.1
25	16	31.0	69.6
31	15	35.0	68.1
32	>20	28.0	68.9
34	15	34.8	63.5
37	16	29.3	67.3
38	15	27.0	65.0
39	15	36.7	61.9
Mean ± SD		31.46 ± 3.2%	65.56 ± 7.1%
NHS		26.8 ± 2.6%	38.5 ± 3.3%

The microtitre plates were coated with MG-Lys-Cu^{2+} modified human DNA (2.5 µg/ml).
NHS: Normal Human Sera.

purity was confirmed by a single homogenous band on SDS-PAGE under non-reducing conditions (data not shown).

Binding of IgG from different diabetes type 1 patients to native and MG-Lys-Cu^{2+} modified human DNA

Purified IgG from Type 1 diabetes patients, were subjected to direct binding ELISA, on a microtitre plate coated with native human DNA and MG-Lys-Cu^{2+} modified human DNA to evaluate the amount required for antigen saturation. The saturation for modified human DNA was obtained at 50 µg/ml of IgG, while for native human antigenic saturation could not be ascertained because of its negligible binding. Therefore, for Type 1 diabetes, IgG concentration was kept constant (50 µg/ml) in all further experiments unless indicated. The binding specificity of the isolated IgG, toward native and MG-Lys-Cu^{2+} modified human DNA, was evaluated by inhibition ELISA. The IgG was mixed with varying amounts of native or MG-Lys-Cu^{2+} modified human DNA (0–20 µg/ml) and incubated for 2 h at 37 °C and overnight

modified human DNA, while with native human DNA it showed 24.4 ± 2.5% mean inhibition. The above results have been summarized in Table 1a.

Purification of IgG from the sera of type 1 diabetes patient

IgG was isolated on a protein A-agarose column from selected high binding sera of Type 1 diabetes patients. The purified IgG eluted as a symmetrical single peak on the affinity column. IgG
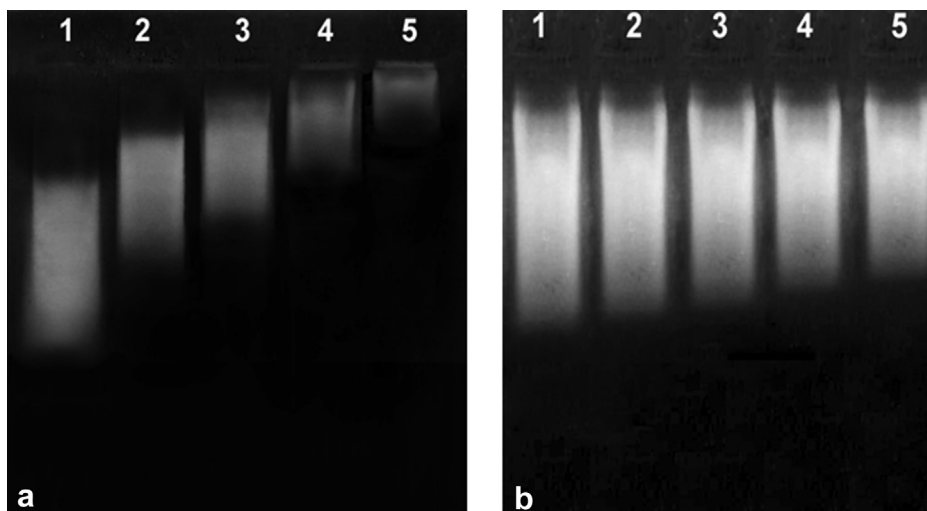
Figure 4. Band shift assay of IgG isolated from diabetes type 1 patient serum with MG-Lys-Cu^{2+} modified human DNA(a) and native human DNA(b). Varying concentrations of IgG were incubated with a constant amount of DNA (0.5 µg) for 2 h at 37 °C and overnight at 4 °C. Electrophoresis was carried out on 0.8% agarose gel for 2 h at 30 mA. Lane 1 contains native (or modified) human DNA while lanes 2–5 contain native or MG-Lys-Cu^{2+} modified human DNA with 20, 40, 60 and 80 µg of IgG from type 1 diabetes patient.

at 4 °C. The observed antibody (IgG) inhibition ranged from 60.1 to 69.6% when modified human DNA was employed as inhibitor, while with the native human DNA it varied from 27 to 36.7%; maximum inhibitor concentration being 20 μg/ml in both the cases. The mean of inhibitions for various sample tested with the MG-Lys-Cu^{2+} modified human DNA was 65.56 ± 7.1%, while with native human DNA, it was 31.46 ± 3.2%. However, under similar experimental conditions normal human subjects showed mean inhibition of 38.5 ± 3.3% with MG-Lys-Cu^{2+} modified human DNA, while with native human DNA it showed 26.8 ± 2.6% mean inhibition. Table 1b summarizes the inhibition data of isolated IgG of diabetes Type 1 group.

Band shift assay

Band shift assay was performed for the visual detection of interaction of native and MG-Lys-Cu^{2+} modified human DNA with purified IgG from type 1 diabetes patients. Equal amount of native and modified DNA samples were incubated with increasing concentrations of IgG for 2 h at 37 °C and overnight at 4 °C. This resulted in a proportional increase in the formation of high molecular weight immune complexes as visualized by retarded mobility and gradually increased band intensity near the wells in agarose gel electrophoresis, exhibiting better recognition of the modified epitopes by the IgG from diabetes type 1 patients (Fig. 4 a & b).

Discussion

Glycation adducts of DNA may have potential as biomarkers since all nucleated cells contain the same DNA content and should reflect the relative level of MG in the target tissue. Reaction of double-stranded DNA with MG or glucose *in vitro* produces primarily N^2-carboxyethyl-2′-deoxyguanosine (CEdG), suggesting to be the likely major adduct formed *in vivo* [20,21]. This implies that CEdG might be a useful biomarker for monitoring oxoaldehyde-induced stress in response to enhanced glycolytic flux or environmental exposure to MG. The preparative HPLC was employed for the synthesis of the standard, carboxyethyl deoxyguanosine (CEdG). The LC-MS was performed to detect the glycated adduct, CEdG formed with the double stranded human DNA. The acid hydrolyzate of MG-Lys-Cu^{2+} glycated human DNA showed an *m/z* value of 338 in the negative ion mode, which is in conformity with the m/z value for standard CEdG. This finding is consistent with an earlier study from our group [15]. Since CEDG has been reported as the major DNA adduct formed as a result of glycation, it could serve as an effective biomarker for the detection of glycation events taking place in our body. It has been reported that the reaction of deoxy-guanosine (dG) with MG proceeds via Amadori pathway [22]. In our case the ESI-MS, mass-spectroscopic data has shown similar results, i.e., the reaction of human DNA with MG-Lys-Cu^{2+} proceeds via the classic Amadori pathway and yields glycation-like products similar to those generated between a nucleoside and a carbohydrate. This is in conformity with the results we obtained for ESI-MS. The ion at *m/z* 341, 679, 268 and 385 is consistent with a [Schiff base + H]$^+$, [Schiff base + H]$^+$ dimer product, dG-H$_2$O and a fragment formed by the degradation of MG reacting with the Schiff base product, or its enaminol or Amadori intermediate respectively.

Increased glycation and, in particular, accumulation of tissue and serum AGEs have an important role in the pathogenesis of diabetic complications. The chemical nature of many AGEs, their synthesis *in vivo* and their precise role in the pathogenesis of complications of diabetes are under intense investigation [1]. Reactive dicarbonyl compounds formed endogenously like, glyoxal, methylglyoxal (MG) and 3-deoxyglucosone, are potent glycating agents having potential role in diabetes and secondary complications associated with it [23]. Glycation by MG is increased disproportionately compared to the increase in glucose concentration in experimental and clinical diabetes [5]. Recently, D-ribose too has gained significance prominence in the glycation of DNA and low density lipo-protein which might result in the pathophysiology of complications associated with diabetes and atherosclerosis [24,25]. The presence of auto-antibodies to more than two dozen auto-antigens have been associated with diabetes type 1 disease, a majority of interest has been directed to islet cell auto-antibodies (ICA), insulin auto-antibodies (IAA), glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like IA-2 auto-antigen [26]. These auto-antibodies, combined with other metabolic and genetic markers, are extremely effective for predicting eventual development of Type 1 diabetes. In view of this, the possible involvement of MG-Lys-Cu^{2+} modified human DNA in diabetes mellitus Type 1 was probed. The binding of circulating auto-antibodies from 40 Type 1 diabetes patients and 20 healthy normal subjects to native and MG-Lys-Cu^{2+} modified human DNA was studied by direct binding ELISA. Of the 40 sera in Type 1 diabetes, 67.5% showed preferentially high binding to MG-Lys-Cu^{2+} modified human DNA as compared to its native analog. No appreciable binding either with native or MG-Lys-Cu^{2+} modified human DNA was observed with serum antibodies from healthy normal subjects. Competition ELISA results showed 22–33% inhibition in the type-I diabetes auto-antibodies binding to native human DNA, whereas 46.9–63.1% inhibition was observed with MG-Lys-Cu^{2+} modified human DNA. These results indicate appreciable recognition of MG-Lys-Cu^{2+} modified human DNA by the auto-antibodies in diabetes (type-I) patients. The binding specificity of the isolated IgG, toward native and MG-Lys-Cu^{2+} modified human DNA was evaluated by competition ELISA. Immunoglobulin G (IgG) from diabetic patients (type-I) recorded an inhibition of 59%–69% with the MG-Lys-Cu^{2+} modified human DNA, while with native human DNA it ranged from 27% to 36%. Appreciably high binding of affinity purified IgG toward MG-Lys-Cu^{2+} modified human DNA, is indicative of the generation of antibodies against RCS modified epitopes on the DNA molecules in diabetes. The strong binding of auto-antibodies from diabetes Type 1 patients to MG-Lys-Cu^{2+} modified human DNA points toward the involvement of modified bases and single strand regions in the disease process. The spontaneous production of auto-antibodies in Type 1 diabetes might be a result of the generation of the antigenic epitopes on the DNA molecules as a result of hyperglycemic condition in the disease. These epitopes are recognized as '*non self*' by the body's immune system, leading to the induction of autoantibodies in diabetes type 1 patients and projecting the glycated DNA as one of the factors eliciting the immune response in diabetes. These autoantibodies may serve as a biomarker for the disease. The presence of auto-antibodies against MG-Lys-Cu^{2+} glycated human DNA in diabetes mellitus patients is suggestive of the involvement of reactive carbonyl species generated epitopes in autoimmune response in diabetes. Alternatively, the experimentally induced antibodies against reactive carbonyl species modified macromolecules may be used for the detection of modified epitopes in diabetes patients.

References

[1] Ahmed N. Advanced glycation endproducts-role in pathology of diabetic complications. Diabetes Res Clin Pract 2006;67:3–21.
[2] Mustafa I, Ahmad S, Dixit K, Moinuddin, Ahmad J, Ali A. Glycated human DNA is a preferred antigen for anti-DNA antibodies in diabetic patients. Diabetes Res Clin Pract 2012;95:98–104.

[3] Thornalley PJ. Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. Cell Mol Biol 1998;44:1013—23.

[4] Stern DM, Yan SD, Yan SF, Schmidt A-M. Receptor for advanced glycation endproducts (RAGE) and the complications of diabetes. Ageing Res Rev 2002;1:1—15.

[5] McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci 1994;87:21—9.

[6] Phillips SA, Thornalley PJ. The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. Eur J Biochem 1993;212(1):101—5.

[7] Chan W, Wu H. Protective effects of curcumin on methylglyoxal-induced oxidative DNA damage and cell injury in human mononuclear cells. Acta Pharmacol Sin 2006;27:1192—8.

[8] Sengupta B, Swenson J. Properties of normal and glycated human hemoglobin in presence and absence of antioxidant. Biochem Biophys Res Commun 2005;334:954—9.

[9] Suarez G, Rajaram R, Oronsky AL, Gawinowicz MA. Nonenzymatic glycation of bovine serum albumin by fructose (fructation). Comparison with the Maillard reaction initiated by glucose. J Biol Chem 1989;264(7):3674—9.

[10] Bucala R, Model P, Russel M, Cerami A. Modification of DNA by glucose 6-phosphate induces DNA rearrangements in an Escherichia coli plasmid. Proc Natl Acad Sci U S A 1985;82:8439—42.

[11] Lee A, Cerami A. Nonenzymatic glycosylation of DNA by reducing sugars. Prog Clin Biol Res 1989;304:291—9.

[12] Dutta U, Cohenford MA, Dain JA. Nonenzymatic glycation of DNA nucleosides with reducing sugars. Anal Biochem 2005;345:171—80.

[13] Ahmad S, Moinuddin, Dixit K, Shahab U, Alam K, Ali A. Genotoxicity and immunogenicity of DNA-advanced glycation end products formed by methylglyoxal and lysine in presence of Cu^{2+}. Biochem Biophys Res Commun 2011;407:568—74.

[14] Ahmad MI, Ahmad S, Moinuddin. Preferential recognition of methylglyoxal modified calf thymus DNA by circulating antibodies in cancer patients. Indian J Biochem Biophys 2011;48:290—6.

[15] Ashraf JM, Arif B, Dixit K, Moinuddin, Alam K. Physicochemical analysis of structural changes in DNA modified with glucose. Int J Biol Macromol 2012;51:604—11.

[16] Ahmad S, Moinuddin, Ali A. Immunological studies on glycated human IgG. Life Sci 2012;90:980—7.

[17] Shahab U, Moinuddin, Ahmad S, Dixit K, Abidi SM, Alam K, et al. Acquired immunogenicity of human DNA damaged by N-hydroxy-N-acetyl-4-aminobiphenyl. IUBMB Life 2012;64:340—5.

[18] Shahab U, Ahmad S, Moinuddin, Dixit K, Habib S, Alam K, et al. Hydroxyl radical modification of Collagen type II increases its Arthritogenicity and immunogenicity. PLoS One 2012;7(2):e31199.

[19] Moinuddin, Dixit K, Ahmad S, Shahab U, Habib S, Naim M, et al. Human DNA damage by the synergistic action of 4-aminobiphenyl and nitric oxide: an immunochemical study. Environ Toxicol 2014;29(5):568—76.

[20] Frischmann M, Bidmon C, Angerer J, Pischetsrieder M. Identification of DNA adducts of methylglyoxal. Chem Res Toxicol 2005;18(10):1586—92.

[21] Papoulis A, al-Abed Y, Bucala R. Identification of N2-(1-carboxyethyl) guanine (CEG) as a guanine advanced glycosylation end product. Biochemistry 1995;34(2):648—55.

[22] Li Y, Cohenford MA, Dutta U, Dain JA. The structural modification of DNA nucleosides by nonenzymatic glycation: an in vitro study based on the reactions of glyoxal and methylglyoxal with 2'-deoxyguanosine. Anal Bioanal Chem 2008;390(2):679—88.

[23] Ahmad S, Moinuddin, Shahab U, Khan MS, Habeeb S, Alam K, et al. Glyco-oxidative damage to human DNA— Neo-antigenic epitopes on DNA molecule could be a possible reason for autoimmune response in type 1 diabetes. Glycobiology 2013;24(3):281—91.

[24] Akhter F, Khan MS, Shahab U, Moinuddin, Ahmad S. Bio-physical characterization of ribose induced glycation: a mechanistic study on DNA perturbations. Int J Biol Macromol 2013;58:206—10.

[25] Ahmad S, Akhter F, Shahab U, Moinuddin, Khan MS. Studies on glycation of human low density lipoprotein: a functional insight into physico-chemical analysis. Int J Biol Macromol 2013;62:167—71.

[26] Pihoker C, Gilliam LK, Hampe CS, Lernmark A. Autoantibodies in diabetes. Diabetes 2005;54(2):S52—61.