An automated Fpg-based FADU method for the detection of oxidative DNA lesions and screening of antioxidants

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Abstract

The oxidation of guanine to 8-oxo-2 -deoxyguanosine (8-oxo-dG) is one of the most abundant and best studied oxidative DNA lesions and is commonly used as a biomarker for oxidative stress. Over the last decades, various methods for the detection of DNA oxidation products have been established and optimized. However, some of them lack sensitivity or are prone to artifact formation, while others are time-consuming, which hampers their application in screening approaches. In this study, we present a formamidopyrimidine glycosylase (Fpg)-based method to detect oxidative lesions in isolated DNA using a modified protocol of the automated version of the fluorimetric detection of alkaline DNA unwinding (FADU) method, initially developed for the measurement of DNA strand breaks (Moreno-Villanueva et al., 2009. BMC Biotechnol. 9, 39). The FADU-Fpg method was validated using a plasmid DNA model, mimicking mitochondrial DNA, and the results were correlated to 8-oxo-dG levels as measured by LC–MS/MS. The FADU-Fpg method can be applied to analyze the potential of compounds to induce DNA strand breaks and oxidative lesions, as exemplified here by treating plasmid DNA with the peroxynitrite-generating molecule Sin-1. Moreover, this method can be used to screen DNA-protective effects of antioxidant substances, as exemplified here for a small-molecule, *i.e.*, uric acid, and a protein, *i.e.*, manganese superoxide dismutase, both of which displayed a dose-dependent protection against the generation of oxidative DNA lesions. In conclusion, the automated FADU-Fpg method offers a rapid and reliable measurement for the detection of peroxynitrite-mediated DNA damage in a cell-free system, rendering it an ideal method for screening the DNA-protective effects of antioxidant compounds.

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1. Introduction

A variety of reactive oxygen and nitrogen species (ROS and RNS) are generated in biological systems, either endogenously from cellular metabolism and inflammatory reactions or exogenously by a variety of chemicals or ionizing radiation (Delaney et al., 2012; Kasai et al., 1986). ROS and RNS can react with DNA molecules, resulting in a wide spectrum of damage products (Delaney et al., 2012; Tretyakova et al., 2012). Due to the low oxidation potential of guanine, deoxyguanosine (dG) oxidation products, such as 8-oxo-2′-deoxyguanosine (8-oxo-dG) and the formamidopyrimidine Fapy-dG, are among the most prominent DNA lesions (Delaney et al., 2012; Neeley and Essigmann, 2006). Although efficient repair mechanisms for oxidative DNA lesions exist, *i.e.*, base excision repair (BER), their formation can affect the efficiency of transcriptional processes (Khobta et al., 2010; Kitsera et al., 2011) and such lesions can be both cytotoxic and mutagenic (Christmann et al., 2003; Delaney et al., 2012; Shibutani et al., 1991). In addition to the nuclear genome, mitochondrial DNA (mtDNA) can be damaged by ROS and RNS generated during mitochondrial respiration (Kazak et al., 2012). Thus, superoxide radicals ($O_2^{-\bullet}$) lacking from the electron transport chain can react with diffusible nitric oxide (NO) produced by nitric oxide synthases to generate $ONOO^-$ and several highly reactive metabolites. In this respect, it was shown that mutations in mtDNA play a role in degenerative diseases of the central nervous and endocrine systems, heart, kidney and skeletal muscle (Wallace et al., 1998, 1999). In general, the assessment of oxidative DNA damage has been widely used as a biomarker of oxidative stress in experimental as well as in epidemiological studies (Dizdaroglu, 2012; Ravanat et al., 2012).

Several methods for the detection of oxidative DNA lesions have been developed, but results vary considerably depending on the different analytical systems and the source of DNA used (Anson et al., 2000; Ravanat, 2012; Rossner and Sram, 2012).

High-performance liquid chromatography coupled with electrochemical detection (HPLC/ECD) has been one of the first methods used for 8-oxo-dG measurement (Floyd et al., 1986; Ravanat, 2012). The method is based on the fact that the low oxidation potential of 8-oxo-dG allows specific and sensitive detection in the one-electron oxidation mode. However, its disadvantages include a risk for the induction of high levels of artifactual lesion and a lack of internal calibration (Ravanat, 2012). Methods based on gas chromatography coupled to mass spectrometry (GC/MS) have a tendency to overestimate 8-oxo-dG levels by 10- to 50-fold, in part due to the acidic conditions of the DNA hydrolysis as well as high temperature during the derivatization step (Ravanat, 2012). Pre-purification of samples by HPLC, or lowering of the derivatization temperature or addition of antioxidants have been suggested to avoid this type of artifact (Halliwell and Dizdaroglu, 1992). At present, one of the most specific and sensitive methods for the detection of a wide spectrum of DNA oxidation products represents reverse phase HPLC coupled ESI tandem mass spectrometry (LC–MS/MS) which does not require derivatization prior to mass spectrometric analysis (Cadet et al., 2002; Mangerich et al., 2012; Ravanat, 2012; Taghizadeh et al., 2008; Tretyakova et al., 2012).

In contrast to chromatographic methods, immunological methods demand little sample preparation and allow the analysis of a panel of samples simultaneously (Rossner and Sram, 2012). For example, ELISA test are widely used as a fast and cheap method for detecting 8-

oxo-dG in human fluids (Cooke et al., 2006). However, the usage of such immuno-chemical techniques remains limited, because of weak antigenicity and chemical selectivity of the antibodies used, as significant cross-reactivity with the undamaged nucleobases has been described (Mitchell et al., 2002; Serrano et al., 1996).

Finally, the comet assay (or single cell electrophoresis assay), alkaline unwinding and alkaline elution techniques can be used to detect a wide spectrum DNA lesions (Gedik and Collins, 2005; Ravanat, 2012; Wood et al., 2010). These methods require use of glycosylases, such as formamidopyrimidine glycosylase (Fpg) or endonuclease III (EndoIII) that specifically convert the oxidized and modified pyrimidine and purine bases into DNA strand breaks, which in turn can be detected in a very sensitive manner (Pflaum et al., 1997; Ravanat, 2012). Different variations of these methods have been developed, including comet-based assays that enable high-throughput assessment of cellular DNA damage (Wood et al., 2010) and methods that combine the comet assay and *in situ* fluorescent hybridization (FISH) to detect oxidized bases at the sequence level (Shaposhnikov et al., 2011). A limitation of glycosylase-based methods is that they rely on the complete enzymatic processing of all damaged bases present and the substrate specificity of the glycosylase (Ravanat, 2012). For example, Fpg is not entirely specific for 8-oxo-dG only, as it was reported to recognize also other oxidation products such as Fapy-dG and Fapy-dA sites (Pouget et al., 2000) as well as alkylation damage (Speit et al., 2004).

Similar to the comet assay, the automated FADU assay allows the quantification of DNA strand breaks in whole cells in a high-throughput manner (Brunner et al., 2012; Debiak et al., 2011; Garm et al., 2013; Kappes et al., 2008; Mangerich et al., 2010; Moreno-Villanueva et al., 2009, 2011). This method is based on a partial denaturation/unwinding of double stranded DNA under time- and temperature-controlled alkaline conditions in a 96-well-plate format using an automated liquid handling device. Subsequently, addition of the fluorescent probe SYBR Green is employed to quantify the state of DNA unwinding, *i.e.*, fluorescence intensities inversely correlated with the number of DNA strand breaks. The objective of the current study was to develop an Fpg-based FADU method to detect oxidative DNA damage in a high through-put manner by adapting of the automated FADU assay and to validate this method by comparing it with results from LC–MS/MS analysis.

2. Materials and methods

All chemicals were of analytical grade and obtained from Sigma–Aldrich, Fluka or Merck.

2.1. FADU-Fpg method

2.1.1. Plasmid DNA treatment—As a DNA model system, a 14-kbp plasmid (pAcHLT-A-His6) was amplified in *Escherichia coli* DH5 cells and extracted using a DNA purification Giga Prep Kit (Qiagen). Each DNA sample was prepared in triplicates containing 104 μg of DNA. Samples were supplemented with uric acid or MnSOD (Ab Frontier) and treated with 100–400 μM freshly prepared Sin-1 (Calbiochem) for 40 min at 30 °C. Samples were then divided into aliquots of 4 μg and 100 μg of DNA for FADU and LC–MS analysis, respectively.

2.1.2. Fpg treatment—Each sample was incubated with 8 U Fpg and NEB1 Buffer (New England Biolabs) for 30 min at 30 °C. Samples were diluted in 280 μl FADU suspension buffer (250 mM *meso*-inositol, 10 mM sodium phosphate, 1 mM MgCl, pH 7.4) and quadruplicates were transferred into a 96-well plate which was positioned into the FADU pipetting robot.

2.1.3. Automated steps of the Fpg-FADU assay—The liquid handling device as well as its positioning have been described previously (Moreno-Villanueva et al., 2009). The original protocol was adapted to optimize the method for the detection of Fpg-generated strand breaks in a cell-free system. The temperature of the cooling device was maintained at 2 °C throughout the entire experiment. First, 70 μl of FADU urea buffer (9 M urea; 10 mM NaOH; 2.5 mM cyclohexyl-diamine-tetraacetate; 0.1% sodium dodecyl sulfate) were dispensed into each well. Immediately thereafter, 70 μl of alkaline buffer (0.425 parts FADU urea buffer in 0.2 M NaOH) was added. The subsequent incubation step of the original protocol was omitted to avoid total unwinding of the plasmid. A volume of 140 μl of neutralization buffer (14 mM -mercaptoethanol; 1 M glucose) was added at a rate of 200 μl/s. Finally, 156 μl of SybrGreen (MoBiTec) diluted 1:8.33 in water was added and samples were mixed by pipetting a volume of 400 μl up and down at a rate of 100 μl/s. Fluorescence intensity was measured in a 96-well-plate fluorescence reader at 492 nm excitation/520 nm emission.

2.2. 8-oxo-dG detection by LC–MS/MS

2.2.1. DNA hydrolysis and dephosphorylation of nucleotides—DNA was desiccated under vacuum and hydrolyzed to nucleosides by a combination of nuclease P1, DNase I, phosphodiesterase I (USB-Affymetrix, Santa Clara, CA), and alkaline phophatase in the presence of deaminase inhibitors and antioxidants as described previously (Taghizadeh et al., 2008). Enzymes were removed by centrifugation at $16,400 \times g$ for 20 min through a 10,000 MW cut-off spin filter (Pall).

2.2.2. Reverse phase HPLC pre-purification—A LC-10AT HPLC system from Shimadzu was used for 8-oxo-dG pre-purification equipped with a Phenomenex Synergi 4-μm Hydro-RP C18 80A (250 mm × 4.6 mm) column. A solvent gradient of acetonitrile in 8 mM ammonium acetate was set at a flow rate 0.7 ml/min (for gradient composition see Suppl. Table 1). The detection was performed using UV–vis spectroscopy at 260 nm. The 8-oxo-dG containing fractions were collected at a retention time of approximately 37–42 min.

2.2.3. LC–MS/MS analysis—8-oxo-dG quantification was performed with an HPLC (Waters 2695 Separations Module) coupled to a triple quadrupole mass spectrometer (Waters Micromass Quattro Micro). A reversed phase Hypersil-Gold column C18 (Thermo Scientific; 150 mm × 2.1 mm; 3 μm particle size) was used and eluted isocratically with 98.5% of H_2O supplemented with 0.1% acetic acid and 1.5% of acetonitrile supplemented with 0.1% acetic acid at a flow rate of 0.3 ml/min. Detailed chromatographic settings are listed in Suppl. Table 2. 22.5 μl of each sample was analyzed in a single LC/MS–MS run. The mass spectrometer was used in the ESI[+] MS/MS mode with settings indicated in Suppl. Table 3. For detection, the multiple reaction monitoring (MRM) mode was used, and transition of m/z 284.0 168.0 for 8-oxo-dG was monitored. All measurements were performed in technical duplicates. The area from one sample was converted into amount in fmol using external calibration curves. The average was calculated from the technical duplicates in fmol.

3. Results

3.1. Analysis of Sin-1-induced DNA damage in a plasmid DNA model

Here we present a novel Fpg-based method to detect oxidative DNA damage in a cell-free system (Fig. 1), using a modified protocol of the FADU assay (Debiak et al., 2011; Moreno-Villanueva et al., 2009, 2011).

A 14-kbp plasmid, mimicking the mtDNA molecule, was treated with the $ONOO^-$ generator 3-morpholinosydnonimine (Sin-1). Sin-1 is thought to mimic the physiological situation in cells as it decomposes to produce equimolar amounts of $NO^\bullet$ and $O_2^{-\bullet}$ and the stable end-product Sin-1C (Fig. 2). This results in a continual flux of $ONOO^-$ over extended periods resulting in oxidative and nitrosative DNA damage (Fig. 2) (Cui et al., 2013; Dedon and Tannenbaum, 2004; Feelisch et al., 1989; Hogg et al., 1992). It was shown previously that the DNA damage profile generated by Sin-1 mainly comprises Fpg-sensitive lesions and to a lesser extend also DNA single strand breaks (Epe et al., 1996). To convert Sin-1-induced oxidative DNA lesions into single-strand break that can be detected by the FADU method, the original FADU protocol was adapted by incubating Sin-1-treated DNA with Fpg (Fig. 1). Thereafter, samples were diluted in a suspension buffer, distributed in a 96-well plate and subjected to the successive automated addition of solutions by the liquid handling device (Fig. 1). Then SYBR Green fluorescence was determined using a 96-well-plate fluorescence reader. The original FADU protocol was further modified to adapt the method for the application to detect Fpg-sensitive DNA lesions. The dilution of the alkaline buffer, the suppression of the alkaline unwinding step and the maintenance of a constant temperature at 2 °C were necessary to prevent complete unwinding of the DNA and to allow a sensitive detection of the Fpg-induced strand breaks. The total hands-on time required for these steps is less than an hour and the number of samples analyzed concurrently is about 20 in quadruplicates. Throughput can be further enhanced by increasing the number of 96-well plates processed at the same time by the pipetting robot.

To determine optimal working concentration of Fpg, samples were incubated with increasing concentrations of Fpg after DNA had been treated with 400 µM Sin-1 (Fig. 3A). The number of Fpg-sensitive lesions detected in the presence of Sin-1 increased until a threshold was reached starting at an Fpg concentration of 0.1 U/µl. This concentration, at which all Fpg-sensitive sites were cleaved, was used for all subsequent FADU experiments. It is important to note that Sin-1 only affected DNA unwinding in the presence of Fpg (Fig. 3B), thus implying that in our system, Sin-1 generates mainly oxidative DNA lesions and does not directly generate DNA single strand breaks in large quantities. This may be related to the fact that $ONOO^-$ reacts with CO_2 to form $ONOOCO_2^-$ (Dedon and Tannenbaum, 2004). While $ONOO^-$ itself, mainly causes deoxyribose oxidation (*i.e.*, strand breaks) and some guanine oxidation, $ONOOCO_2^-$ causes mainly guanine oxidation (Dedon and Tannenbaum, 2004). It is likely that the Sin-1 concentrations as used in our experimental setting are not using up the dissolved CO_2 in the buffer, resulting in the generation of $ONOOCO_2^-$. This information is of high relevance as other oxidizing agents, such as H_2O_2, have been shown to directly generate DNA single strand breaks alongside to oxidative damage, rendering the specific detection of oxidative DNA lesions in these cases particularly biased (unpublished observation).

To study the effect of increasing Sin-1 concentrations on the generation of oxidative DNA lesions, plasmid DNA was treated with Sin-1 concentrations up to 400 µM. Fpg-sensitive DNA lesions significantly increased with Sin-1 concentrations in a dose-dependent manner (Fig. 4A). It was an essential part of this work to validate the FADU-Fpg method by comparing it to another state-of-the-art analytical method used for the detection of DNA oxidation products. To this end, 8-oxo-dG levels were analyzed in the same samples in parallel by LC–MS/MS. A similar approach was used previously to calibrate an Fpg-based Comet assay (Pouget et al., 2000). Previous studies demonstrated that 8-oxo-dG lesions make up of 75% of the total number Sin-1-induced Fpg-sensitive DNA damage sites (Epe et al., 1996), suggesting that 8-oxo-dG formation gives a conservative estimate for the number of Fpg-sensitive guanine oxidation events. In our study basal levels of 8-oxo-dG, as determined by LC–MS/MS, were in the range of 1–10 lesions per 10^6 bp which is consistent with published reference values (Ravanat, 2012; Taghizadeh et al., 2008). The resulting

curve of Sin-1-induced 8-oxo-dG lesions confirms the dose-dependent increase of oxidative lesions observed in the FADU-Fpg method (Fig. 4B). Results obtained from the FADU-Fpg and the LC–MS/MS analysis showed similar sensitivity and a highly significant positive correlation (r^2 of 0.998) (Fig. 4C) indicating that the FADU-Fpg method indeed detects oxidative DNA lesions in a sensitive and dose dependent manner.

3.2. Scavenging of Sin-1 oxidation in a plasmid DNA model

The automated FADU-Fpg method requires little sample preparation and offers a rapid and reliable measurement of oxidative lesions, thus making it a suitable method for screening the antioxidant properties of enzymes and small molecules.

To study the antioxidant properties of MnSOD, which catalyze the dismutation of superoxide into oxygen and hydrogenperoxide, plasmid DNA was supplemented with increasing concentrations of MnSOD (0.94–75 ng/μl) prior to Sin-1 incubation. Both FADU-Fpg and LC–MS/MS measurements highlight the ability of MnSOD to scavenge $ONOO^-$ and concur in demonstrating a dose-dependent protection of the DNA against Sin-1-induced oxidation by MnSOD (Fig. 5A and B). Again, a highly positive correlation between results from both methods was observed, with $r^2 = 0.89$ (Fig. 5C), however, the FADU-Fpg method showed higher reproducibility than LC–MS/MS measurements.

Uric acid is produced from xanthine by xanthine oxidase. It is known to be a powerful antioxidant that is abundantly present in human blood (Ames et al., 1981). After a pre-incubation of plasmid DNA with uric acid in concentrations up to 100 μM, samples were treated with 100 μM Sin-1. Both FADU-Fpg and LC–MS/MS measurements revealed a dose-dependent decrease in Sin-1-induced oxidative DNA lesions starting at a uric acid concentration of 25 μM (Fig. 6A and B). Complete protection of DNA required uric acid concentrations above 50 μM. Values measured by both methods again correlated well with an $r^2 = 0.96$ (Fig. 6C).

4. Discussion

The main drawback of available Fpg-based and LC–MS/MS-based methods is the requirement of elaborate sample preparation and processing steps. We have been able to overcome this limitation by establishing an Fpg-based method for detecting oxidative DNA damage lesions in isolated DNA on the basis of the high-throughput FADU method (Brunner et al., 2012; Debiak et al., 2011; Garm et al., 2013; Kappes et al., 2008; Mangerich et al., 2010; Moreno-Villanueva et al., 2009, 2011). The FADU-Fpg method was validated using a plasmid DNA model mimicking mtDNA in combination with the $ONOO^-$ generator Sin-1 (Fig. 2). An Fpg pre-incubation step has been added to the original FADU protocol that allows the detection of oxidative DNA lesions, by generating strand breaks that can be detected by the FADU method. We have controlled the validity of the method by measuring 8-oxo-dG levels using LC–MS/MS analysis which allows unambiguous chemical selectivity (Taghizadeh et al., 2008).

A notable advantage of the modified FADU-Fpg method over the HPLC-based methods is the quantity of DNA necessary for the analysis. While 25–50 μg is required for LC–MS/MS analysis, only 1 μg of plasmid DNA per measurement was sufficient for the FADU-Fpg method. Moreover, the LC–MS/MS-based method is unfavorable for screening purposes, because it is rather costly and time-consuming. In particular, samples must undergo a number of critical processing steps, *i.e.*, digestion, dephosphorylation, filtering, vacuum-concentration, pre-purification by HPLC, and analyte detection by LC–MS/MS. Also, samples are purified and analyzed sequentially by HPLC and LC–MS/MS, demanding additional time. For a number of 20 samples measured in quadruplicates, a time period of

around 3 days is required. In contrast, the same amount of samples can be measured with the FADU-Fpg method in less than half a day. The fact that in the FADU-Fpg method many samples can be processed in a 96-well plate simultaneously by the automated liquid handling device not only increases throughput, but also ensures equal treatment of samples.

Despite the reported approximate specificity of Fpg toward DNA oxidation and alkylation damage (Pouget et al., 2000; Speit et al., 2004), the side-by-side comparison of the FADU-Fpg and the LC–MS/MS methods in our study resulted in highly significant positive correlations of Fpg-sensitive DNA lesions and 8-oxo-dG levels (Figs. 4–6). This strongly indicates the ability of the FADU-Fpg method to detect oxidative DNA damage.

One important limitation of our method is that, so far, it cannot be applied to determine oxidative DNA damage in cells, due to high concentrations of urea in the FADU cell lysis buffer, which is necessary for complete cell lysis, but which interferes with Fpg activity. Moreover, current methods used for extraction of genomic and mtDNA from cells introduce significant levels of DNA strand breaks, which is incompatible with FADU-Fpg measurements. For this reason, the FADU-Fpg method is currently restricted to the plasmid DNA model as reported in this study. Further optimization and adaptations of the method are indicated to overcome these limitations.

On the other hand, the FADU-Fpg method in its current state is a simple, rapid and reliable high-throughput alternative for measuring relative levels of oxidative DNA damage in plasmid DNA. A comparable technique that has been used for many years uses isolated DNA from the bacteriophage PM2 in combination with DNA glycosylases and electrophoretic separation of supercoiled, circular and linear DNA (Epe et al., 1993, 1996). This method allows easy and reliable detection of a wide range of DNA lesions, however in its present form, is not available in an automated version. As an alternative to this method, the FADU-Fpg method is particularly suitable for screening purposes, *e.g.*, to study protective effects of antioxidant substances, as our experiments with MnSOD and uric acid demonstrate (Figs. 5 and 6). The protective effects of these compounds follow complex chemical mechanisms: While uric acid acts as a general scavenger of ROS (Ames et al., 1981), the protective effect of MnSOD may be related to reduced decomposition of Sin-1, due to MnSOD-mediated scavenging of superoxide (Feelisch et al., 1989), or direct DNA protective effects by binding of MnSOD to DNA (Kienhofer et al., 2009).

Recently the value of the FADU-Fpg method in toxicological and pharmaceutical research has been demonstrated in a study in which the biochemical mechanisms of the neuroprotective and anti-inflammatory effects of minocycline, a semisynthetic derivative of tetracycline, were investigated (Schildknecht et al., 2011). In this study, the FADU-Fpg method, which is described and validated here in detail, was used to determine the peroxynitrite-scavenging properties of minocycline. The low effective concentration of 5 µM for 50% inhibition determined in that study corresponds with standard concentrations present in brain after repeated oral intake, as determined in clinical studies (Schildknecht et al., 2011).

In conclusion, the FADU-Fpg method combines the sensitivity and reliability of an Fpg-based assay for the detection of oxidative DNA lesions with the decreased operation time and high throughput of an automated procedure. For this reason, the FADU-Fpg method represents a useful tool for screening DNA-damaging and antioxidant properties of substances in toxicology, pharmacology, and in the food industry, where the development of nutraceuticals is currently of high priority.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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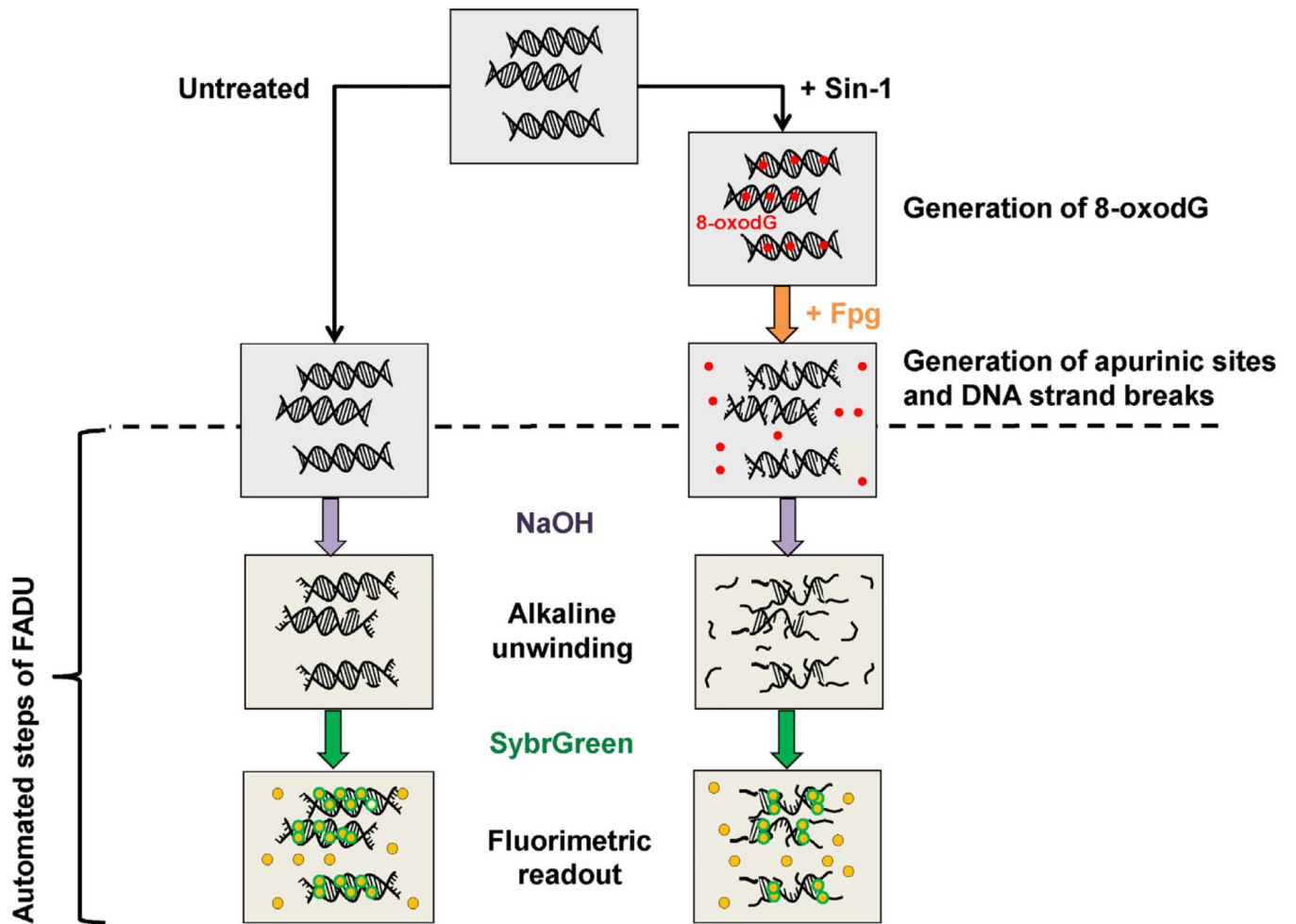
Fig. 1.
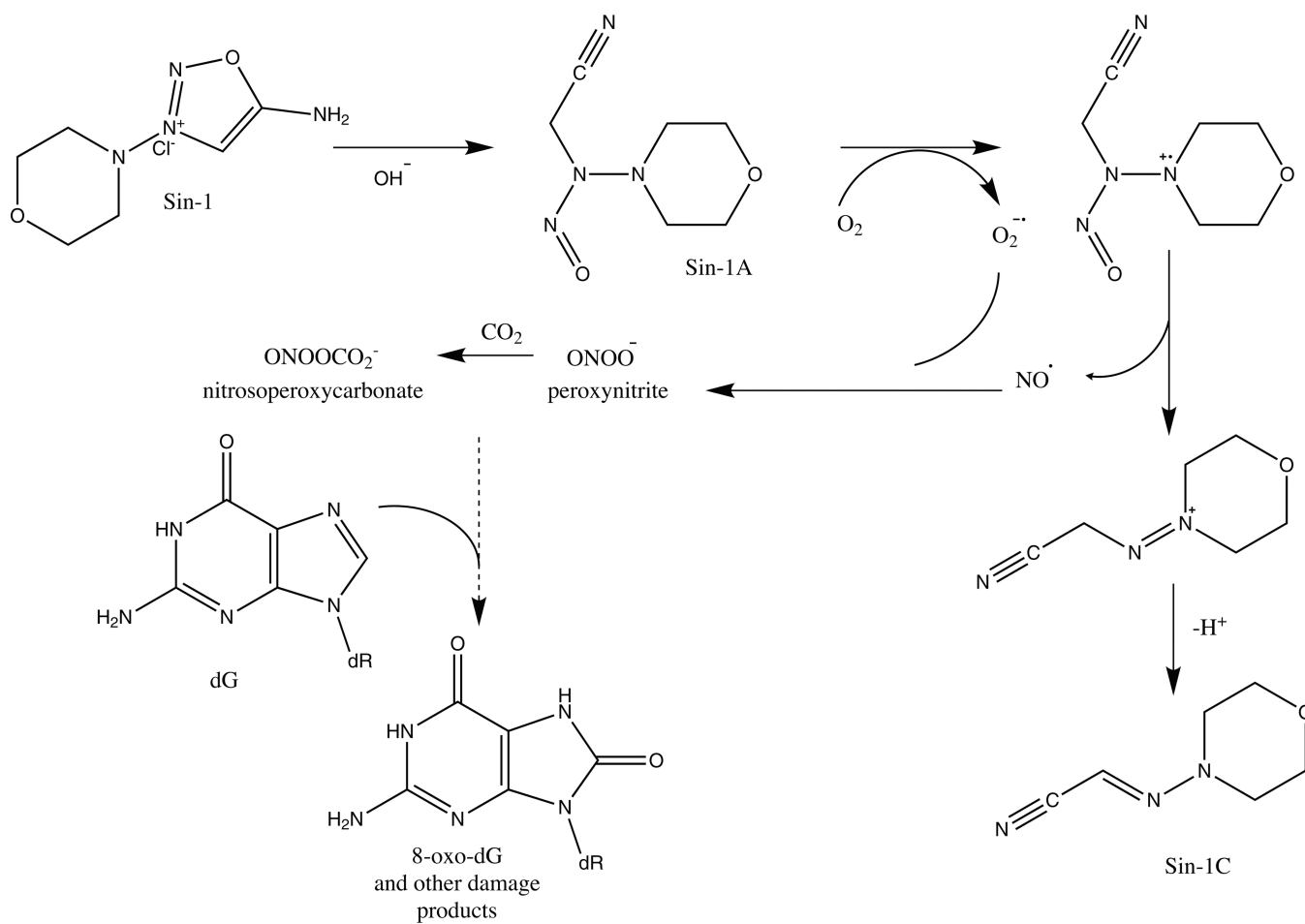Workflow of the FADU-Fpg method.

Fig. 2.
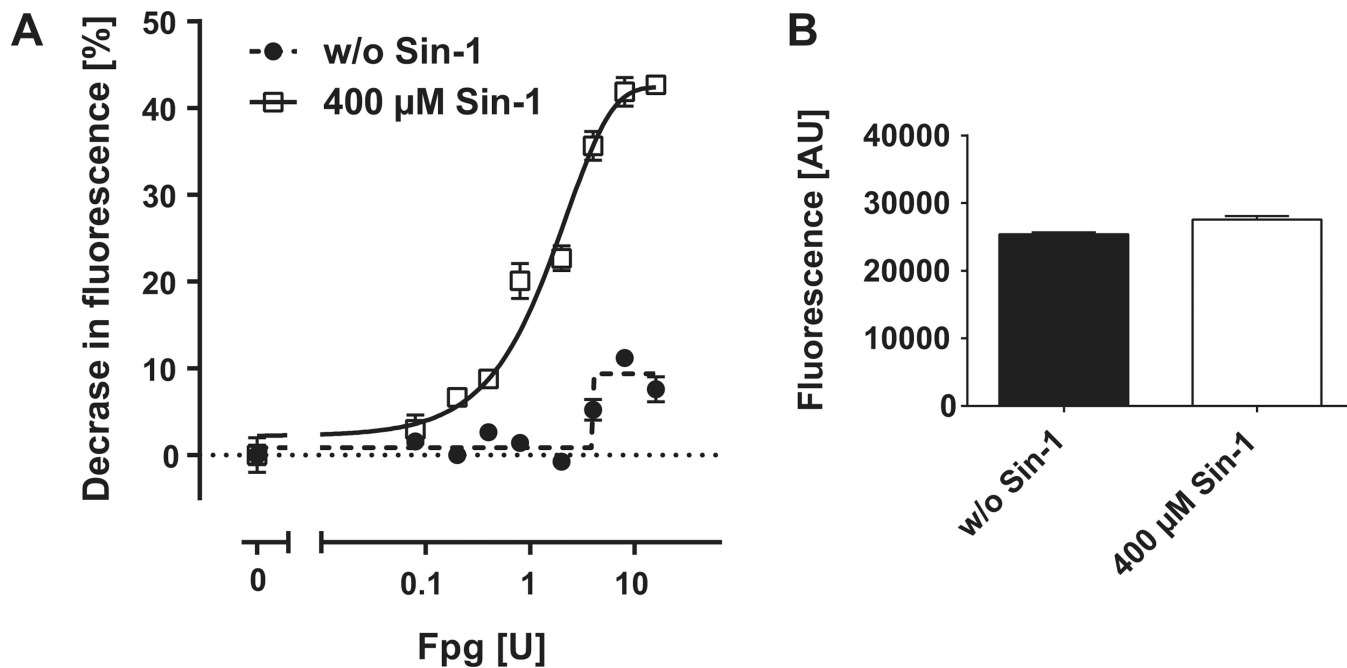Sin-1-induced formation of 8-oxo-dG in DNA.

Fig. 3.
(A) Fpg treatment leads to dose-dependent strand break formation in plasmid DNA treated with Sin-1. Plasmid DNA was treated with or without 400 μM Sin-1 and then incubated with increasing concentrations of Fpg (4–800 mU/μl). (B) Samples treated without or with 400 μM Sin-1 (without Fpg incubation). Experiments were performed in quadruplicates and data are expressed as means ± SEM.
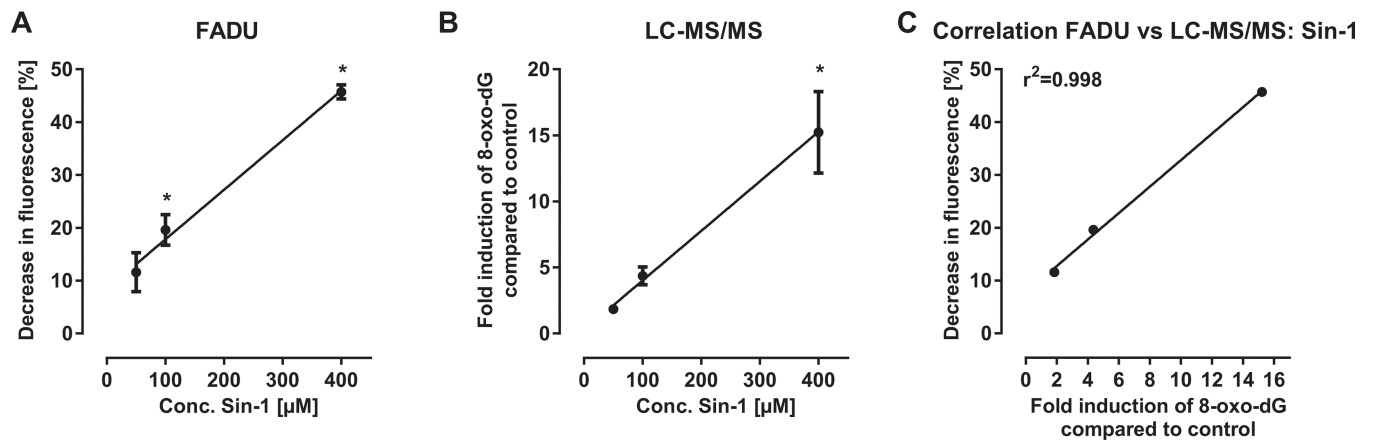
Fig. 4.
Sin-1 induces Fpg-sensitive DNA lesions and 8-oxo-dG in plasmid DNA in a dose-dependent manner. Plasmid DNA was treated with increase in concentrations of Sin-1 (50–400 μM). DNA damage was analyzed (A) by FADU-Fpg and (B) by LC–MS/MS (8-oxo-dG). (C) A linear regression analysis was performed. Samples of each experiment were distributed into aliquots for FADU-Fpg and LC–MS/MS measurements, respectively. Data represent means ± SEM of 3 independent experiments. FADU experiments were performed in technical quadruplicates, LC–MS/MS measurements in technical duplicates. r^2 represents the linear regression coefficient. Statistical analysis was performed by one-way ANOVA test followed by Bonferroni's post test, $*P < 0.05$.
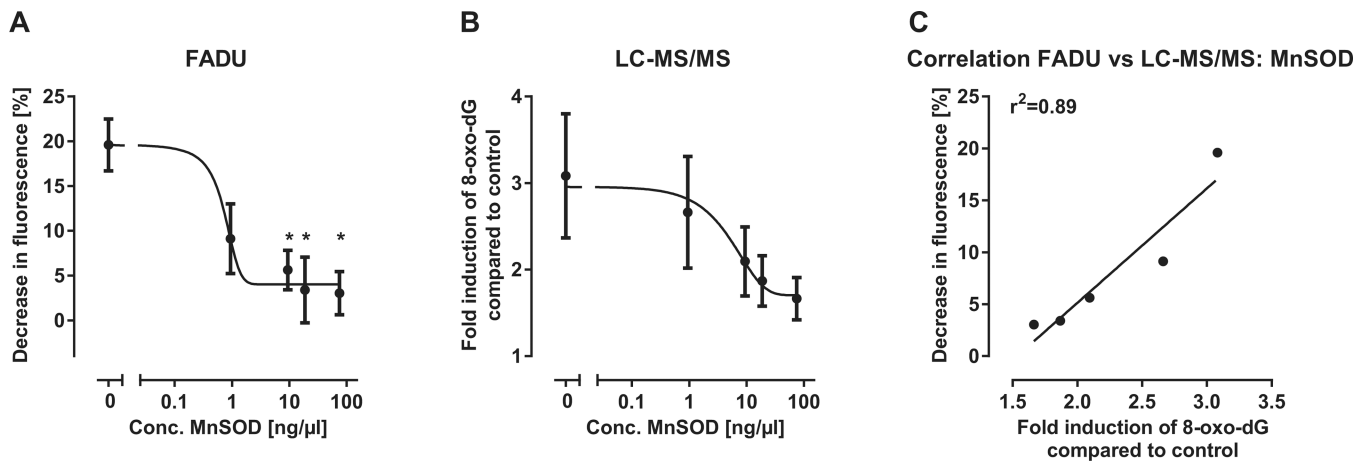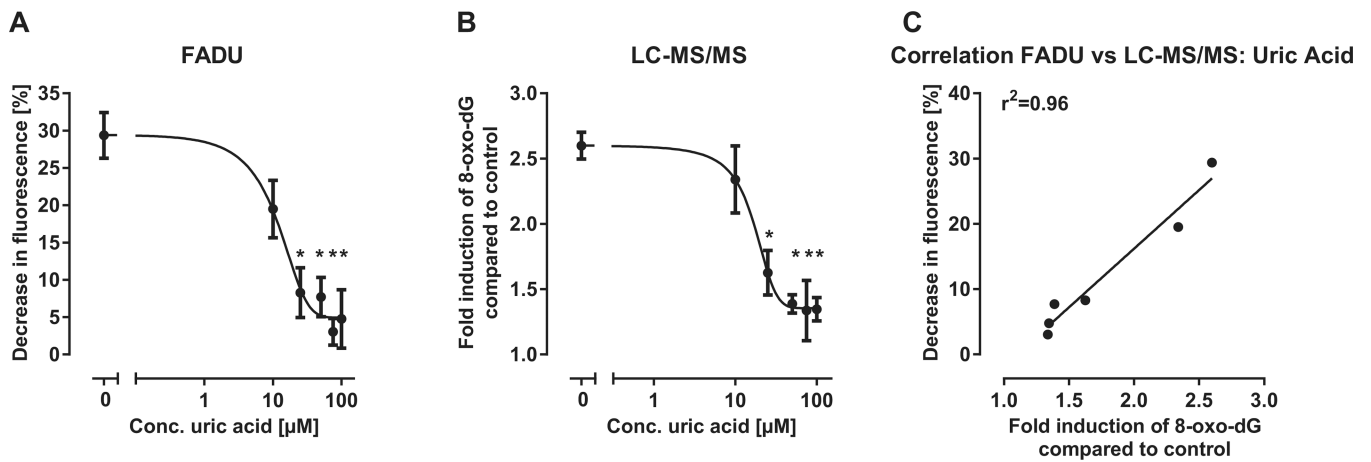
Fig. 5.
MnSOD protects DNA against Sin-1-induced DNA damage in a dose dependent manner. Plasmid DNA was treated with 100 μM Sin-1 and supplemented with increasing concentrations of MnSOD. Oxidative damage was analyzed (A) by FADU-Fpg and (B) by LC–MS/MS (8-oxo-dG). (C) A linear regression analysis was performed. Samples of each experiment were divided into aliquots for FADU-Fpg and LC–MS/MS measurements. Data represent means ± SEM of 3 independent experiments. FADU-Fpg experiments were performed in technical quadruplicates, LC–MS/MS measurements in technical duplicates. r^2 represents the linear regression coefficient. Statistical analysis was performed by one-way ANOVA test followed by Bonferroni's post test, $*P < 0.05$.

Fig. 6.
Uric acid protects DNA against Sin-1-induced DNA damage in a dose-dependent manner. Plasmid DNA was treated with 100 μM Sin-1 and supplemented with increasing concentrations of uric acid. Oxidative damage was analyzed (A) by FADU-Fpg and (B) by LC–MS/MS (8-oxo-dG). (C) A linear regression analysis was performed. Samples of each experiment were divided into aliquots for FADU-Fpg and LC–MS/MS measurements. Data represent means ± SEM of 3 independent experiments. FADU-Fpg experiments were performed in technical quadruplicates, LC–MS/MS measurements in technical duplicates. r^2 represents the linear regression coefficient. Statistical analysis was performed by one-way ANOVA test followed by Bonferroni's post test, $*P < 0.05$.