Quantitative Detection of Benzo[a]pyrene Dioleoperoxide–DNA Adducts by Cryogenic Laser Induced Fluorescence

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In the present report, we describe a fluorescence-based method capable of measuring benzo[a]pyrene dioleoperoxide (BPDE) adducts in intact genomic DNA, with a sensitivity of a few hundreds copies per cell. The assay is based on cryogenic laser-induced fluorescence technology at liquid nitrogen temperatures, coupled with an intensified charge-coupled device camera, and incorporates several enhancements to existing methodologies. One important modification was the incorporation of terbium(III) nitrate pentahydrate, Tb(NO₃)₃, as an internal fluorescence standard to correct for differences in light scattering and fluctuations in instrument parameters. Since the fluorescence spectrum of Tb(NO₃)₃ does not overlap with those of BPDE–DNA adducts, use of this lanthanide salt markedly improved the sensitivity of cryogenic laser-induced fluorescence. The limit of quantification of the assay is 6.4 BPDE–DNA adducts/10⁸ nucleotides, or 776 adducts/cell, using 22.5 μg of genomic DNA. This assay is rapid, highly sensitive, and economical and has been applied to monitor DNA adduct levels as a function of time after exposure to BPDE in repair-competent human lymphoblastoid AHH-1 and TK6 cells.

Introduction

Human tissues are replete with DNA adducts from endogenous and exogenous sources (1). Induction of biological damage by environmental polycyclic aromatic hydrocarbons (PAHs) has long been suspected as a means by which cancers are induced or accelerated in humans. The level of PAH adducts in human tissues has been variously estimated to lie between 100 and 32,000 adducts/cell (0.8–267 per 108 nucleotides) (2–4). Since the pattern of human exposure in most settings involves repeated exposure at relatively low concentrations, long-term low-dose (LTLD) experiments were designed to study the genetic effects of carcinogens at concentrations relevant to human exposure (5–7). LTLD experiments involve continuous treatment of large cell cultures for up to 20 days, with repeated sampling to determine DNA adduct levels and the mutant fraction as a function of time after exposure. These experiments are technically very challenging to perform, since they entail low mutation rates, low DNA adduct levels, and continuous growth of large scale cell cultures without antibiotics for weeks. Monitoring of mutation rates assays in LTLD has typically made use of the established forward mutation assays at HPRT and TK loci. However, detection of the low level of PAH adducts expected in human cells treated with low levels of these chemicals has been problematic. Thus, there was a need for a highly sensitive and economical assay capable of detecting DNA adducts at conditions that mimic human exposures.

Previously, we have been able to obtain data for mutagenic potency of mutagens in a range of concentrations that induce mutations at a rate comparable to the rate of spontaneous mutation in human lymphoblastoid cells. Using tritiated benzo[a]pyrene ([3H]BaP) at a concentration of 100 nM in 4 h pulse exposures, Danheiser et al. (6) found that the level of DNA adduction was approximately 1500 adducts/cell (or 12.5 adducts/10⁸ nucleotides). For the HPRT gene, the rate of mutation was approximately 2.8 times the spontaneous mutation rate. Therefore, an assay with a sensitivity of about 1000 PAH adducts/cell (or 8.3/10⁸ nucleotides) is needed for studies of mutagen at doses representing most human exposures.

Cryogenic laser-induced fluorescence (LIF) spectroscopy is among the most sensitive methods to obtain both qualitative and quantitative information about a compound and has been a valuable tool for the analysis of DNA adducts (8, 9). At cryogenic temperatures the fluorescence peaks are much narrower than those commonly observed at room temperatures. Fluorescence line narrowing spectroscopy (FLNS) (S1 → S₀) at 2 K and fluorescence nonline narrowing spectroscopy (FNLS) (S₂ → S₀) can be used for identifying and for quantifying DNA adducts, respectively. Fluorescence line narrowing spectroscopy (FLNS) results from the excitation of electrons into the first excited state, S₁, and is highly specific. This methodology has been widely applied to the identification of PAH–DNA adducts since different fingerprints were generated for structurally similar PAH–DNA adducts.

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Abbreviations: BaP, benzo[a]pyrene; BPDE, benzo[a]pyrene-7,10-dihydrodiol-7,10-epoxide(anti); EG, ethylene glycol; FLNS, fluorescence nonline narrowing spectroscopy; FNLNS, fluorescence nonline narrowing spectroscopy; ICCD, intensified charge-coupled device; LIF, laser-induced fluorescence; LTLD, long-term low-dose; N₄dGp, 7,8,9,10-tetrahydrobenz[a]pyrene 7,8,9,10-tetrahydrobenz[a]pyrene PAHs, polycyclic aromatic hydrocarbons.
Adduct characterization was accomplished by comparison with synthetic adduct standards. Recently, capillary electrophoresis or HPLC, interfaced with FLNLS was used for the separation and spectral characterization of closely related PAH analytes including stereoisomeric forms (10–17). However, kinetic studies on large numbers of samples at liquid helium temperatures can be very expensive. Due to the increased vibrational level density, as one goes into the $S_2$ state, the line narrowing effects are reduced and fluorescence online narrowing spectroscopy (FNLSNS) is obtained. The fluorescence spectra provided by FLNLS at liquid nitrogen temperatures are expected to be broader with less specific structural features. Studies have shown that the intense fluorescence peak resulting from $S_2$ excitation provides greater sensitivity for quantification of low level of pyrolyl adducts including BPDE adducts (18). FNLSNS ($S_2 \rightarrow S_0$) spectroscopy was chosen for the quantification of BPDE adducts in the present experiment for its high sensitivity and low cost.

The cryogenic laser-excited fluorescence spectra of (±)-anti-BPDE–DNA adducts can be obtained with $\lambda_{ex} = 346$ nm for $S_2$ excitation (8, 19). The fluorescence from (±)-anti-BPDE-calf thymus DNA adducts increase in intensity by a factor of 20 or more as the temperature is lowered from ambient to 77–130 K (18, 20–23). This effect facilitates the quantification of BPDE–DNA adduct levels in cellular DNA and also makes it possible to use intact DNA in the assay, eliminating the tedious and possible error causing steps of adduct hydrolysis, enrichment and cleanup.

In the present study, we enhanced the existing technology of cryogenic laser-induced fluorescence at liquid nitrogen temperatures, coupled with an intensified charge-coupled device (ICCD) camera, and applied this methodology to the quantification of (±)-(anti)benzo[a]pyrene diolepoxide (BPDE) DNA adducts. An important modification was the incorporation of terbium(III) nitrate pentahydrate, Tb(NO$_3$)$_3$, as an internal fluorescence standard to correct for differences in light scattering and fluctuations in instrument parameters. Since the fluorescence spectrum of Tb(NO$_3$)$_3$ does not overlap with those of BPDE–DNA adducts the use of this lanthanide salt markedly improved the sensitivity of the method. Our improved method is a rapid, highly sensitive, and economical analytical technique, which can be used to measure BPDE–DNA adduct levels directly in isolated genomic DNA. The limit of quantification for BPDE–DNA adducts was 3.3 fmol with a signal-to-noise ratio of 46, which corresponded to 6.4 adducts/10$^8$ nucleotides.

The human lymphoblastoid cell line RPMI 1640 medium supplemented with 5% horse serum, and ethylene glycol were obtained from Sigma (St Louis, MO). Ethylene glycol was purified by HPLC using C18 column and then stored at 4 °C until use. Anhydrous DMSO (99.997% purity) was purchased from Aldrich (Milwaukee, WI). Microcentrifugal filter devices were purchased from Millipore (Bedford, MA). 

Preparation of Human Cellular DNA Modified by BPDE. TK6 cells (24) were derived from the parental human lymphoblastoid line HH4 and heterozygous for thymidine kinase (TK), an enzyme which phosphorylates thymidine and its analogues in an ATP-dependent reaction. The AHH-1 TK$^{-/-}$ cell line (25–27) has aryl hydrocarbon hydroxylase (AHH), a member of P4501A1 activity and was isolated from the human B-lymphoblastoid cell line RPMI-1788. It is known that AHH-1 cells can metabolically activate nitrosamines, PAHs, and mycotoxins (28).

Both AHH-1 cells and TK6 cells are anchorage independent and grow in both stationary and stirred cultures with doubling times ranging from 16 to 24 h. Cells were counted using a Coulter counter. Cells were maintained in exponential growth by daily dilution in RPMI 1640 medium supplemented with 5% donor horse serum. In this project, cells were grown in a spinner flask to allow for gentle stirring. Stirred cultures were maintained in 37 °C incubators with a 5% CO$_2$ atmosphere. Since AHH-1 cells are very sensitive to unknown serum factors, screening of serum batches was necessary in order to sustain the cell’s exponential growth.

We compared the kinetics of DNA adduct removal in AHH-1 and TK6 cells with a single dose of BPDE. AHH-1 cells or TK6 cells (at 5 x 10$^5$ cells/mL, 4000 mL) were exposed to BPDE at 10 ng/mL. Duplicate cultures were used for each cell line. Aliquots (200 mL) of cells were removed from each culture at 15 min, 30 min, 45 min, 2 h, 4 h, 6 h, 10 h, 12 h, 24 h, and 36 h after adding BPDE. Cells were centrifuged and resuspended in 1 mL of fresh medium plus 10% DMSO and then placed in a liquid nitrogen freezer to await DNA isolation at a later date for adduct level analysis. Cell count at each time point was taken to monitor the growth of the culture during the treatment.

Preparation of Internal Standard of Human Cellular DNA Modified by [14C]BPDE. TK6 cells were treated with [14C]BPDE (48.2 mCi/mmol or 1.07 x 10$^4$ DPM/mol) at a toxic dose of 63 ng/mL. Then cells were centrifuged and DNA were isolated and cleaned up with Qiagen columns.

DNA Isolation and Purification. A reliable method that allows for the rapid and efficient extraction of intact DNA from cells without contributing fluorescent contaminants is described as follows. DNA was extracted from cells using Qiagen columns, followed by further purification by microdialysis with Microcentrifugal devices.

DNA from up to 10$^8$ cultured cells was isolated by a QIAamp Blood Maxi Kit. With a suitable centrifuge rotor, 12 samples were prepared simultaneously. The size of genomic DNA obtained ranged from 10 to 50 kb. Absorbance was recorded at 260 and 280 nm to determine DNA concentration and purity. The A$_{260}$/A$_{280}$ ratio was between 1.8 and 1.9 for all the samples isolated. DNA was further purified and concentrated using Microcentrifugal filter devices (Millipore) after isolation from cultured cells by Qiagen column. Microcentrifugal filter devices employ low-binding, hydrophilic regenerated cellulose membrane. The membrane has low-adsorption characteristics, which allows usually high recovery rate, 78–95% of the sample in this experiment.

Microcentrifugal filter devices were first prerinse with 400 µL of distilled and deionized water for three times to cleaning trace contaminants on the membranes. After prerinising, about 400–600 µL of DNA solution was loaded on the filter
devices and spun at 14000g. Macromolecules such as DNA remain on the filter, and small molecules such as water, salt, etc., were filtered through. Then the assembly was removed from centrifuge. The sample reservoir with the filter was removed from the vial and placed upside down in a new vial, and then spun for 3 min at 1000g to transfer the concentrate to the vial. Typical processing time was 20 min. This procedure removed some contaminants that originated from either cells or DNA isolation procedures, significantly decreasing background of laser-induced fluorescence of intact DNA from untreated cells.

Instrumentation. The instrumentation was modified from the original design (29). Laser light (1064 nm) was generated with a Nd:YAG laser (Quanta-Ray DCR-11) whose second harmonic at 532 nm was generated a dye laser (Spectra Physics model PDL-3). The output of the dye laser was doubled using a KDP crystal, to generate laser wavelengths in the range of 300--400 nm. Typical pulse energy was in the range of 0.1 mJ to 1 mJ/pulse at a repetition rate of 10 Hz and a pulse width of 5 ns. To increase the fluorescence signal, a cylindrical lens was placed before the sample tube allowing the laser to be focused onto the sample in the shape of a line rather than a spot. As a result, the volume of samples exposed to the laser increased from 0.5 to 2 cm in diameter, with a commensurate 4-fold increase in the fluorescence signal. The laser light was focused onto the sample coded to 77--120 K in a continuous flow liquid nitrogen optical cryostat (Oxford Instruments model CF-1204) by a 10 cm focal length cylindrical lens. With the help of a PID-type (Oxford ITC4) controller, the temperature of the cryostat could be maintained stable in the range of 77--200 K using a slow, continuous flow of liquid nitrogen. Fluorescence emission from the sample was collected at right angles by a pair of lens in which the collection lens is a 5 cm focal length culminating lenses and then focused into a 0.32 m spectrograph using a 15 cm focal length lens, matched to the F-number of the spectrometer. An LPF 350 filter was used to block any wavelengths below 360 nm. The fluorescence was detected with an ICCD-array detector (Princeton Instruments IC51-1224 MG-E with a Princeton Instruments ST-130 controller) with appropriate software on an IBM-compatible PC.

Cryogenic Laser-Induced Fluorescence Spectroscopy To Detect PAH Adducts. In each experiment, 22.5 μg of [14C]-BPDE-DNA (30 μL × 0.75 μg/μL) was mixed in fused silica capillary with 30 μL of purified ethylene glycol (EG) to give 50% mixtures of EG/water. This matrix allows for uniform and crystal-free freezing at cryogenic temperatures as low as 120 K. Ethylene glycol (EG) was spiked with terbium(III) nitrate pentahydrate Tb(NO₃)₃ at a fixed concentration prior to mixing with DNA. The DNA/EG mixture (60 μL) was placed in a 1.5 mm internal diameter fused silica capillary and sonicated for 8 min to remove dissolved gases. The sample was then cooled to 120 K by liquid nitrogen in the optical cryostat, and the laser-induced fluorescence spectrum was measured using an ICCD detector. The fluorescence peak areas were calculated using GRAMS/32 software and then normalized with respect to the area of the strongest peak at 549 nm of Tb(NO₃)₃ internal standard. The final DNA adduct level was obtained by using standard curves from [14C]BPDE treated TK6 cells. The amount of BPDE DNA adducts at various concentrations or time of exposure in treated AHH-1 and TK6 cells were measured using this method.

Results

Development and Validation of High Sensitivity Cryogenic LIF (FNLNS). The spectrum of 7R,8S,9S-trihydroxy-10R-(N²-deoxyguanosyl-3'-phosphate)-7,8,9,10-tetrahydrobenzo[a]pyrene (N²-dGp) standard at 120K. ex = 346 nm. The peaks are at 380, 398, and 419 nm.

![Figure 1](Image 350x317 to 525x511)  
**Figure 1.** FNLN spectrum of 7R,8S,9S-trihydroxy-10R-(N²-deoxyguanosyl-3'-phosphate)-7,8,9,10-tetrahydrobenzo[a]pyrene (N²-dGp) standard at 120K. ex = 346 nm. The peaks are at 380, 398, and 419 nm.

![Figure 2](Image 358x561 to 518x744)  
**Figure 2.** Fluorescence emission spectra from a series of dilutions of [14C]BPDE adducted TK6 DNA. The curves from lowest intensity to highest intensity represent background solvent control, DNA control from untreated TK6 cells, 2, 5.2, 15.5, and 20.7 × 10⁶ adducts from 22.5 μg of DNA (30 μL × 0.75 μg/μL), which corresponds to 776, 2070, 6212, and 8282 adducts/cell, respectively.

obtained from N²-dGp were similar to those of the spectra of BPDE adducted TK6 DNA (Figure 2). These findings suggested that this FNLNS method was unable to distinguish among BPDE-dA, BPDE-dG, and various diastereoisomeric forms, and detected a combination of all BPDE adducts. When compared to the published spectra of BPDE-DNA adducts obtained using an excitation wavelength of λex = 346 nm at 77 K, our spectra had relatively lower peak height at 380 nm, the highest energy side (19). Since our apparatus included a filter to block any wavelengths below 360 nm, truncation of the (0,0)-band on the high-energy side was expected. However, since the filter was fixed in all spectral determinations of standard and unknown samples, the filtering did not affect the measurements of BPDE-DNA adducts.

The cryogenic LIF instrument was calibrated using ¹⁴C-labeled BPDE-DNA adducts. The level of [¹⁴C]-BPDE-DNA was 2624 ± 2.6 DPM/mg DNA, as determined by scintillation counting. For genomic DNA this
level corresponds to 24.52 ± 0.024 pmol/mg of DNA or 132 408 ± 132 [14C]BPDE adducts/cell. Serial dilutions of the [14C]BPDE-DNA stock were prepared for calibration experiments.

To measure BPDE-DNA adducts at both high and low levels in genomic DNA following short-term high-dose and long-term low-dose exposures, we generated two fluorescence standard curves. A calibration curve covered concentrations from 5 × 10^9 to 82 × 10^9 adducts in 22.5 µg of genomic DNA, corresponding to 2000 adducts/cell to 33 000 adducts/cell. Adduct level was determined by scintillation counting [14C]BPDE-DNA. A total of 22.5 µg (30 µL × 0.75 µg/µL) of DNA was loaded and 500 µM Tb(NO3)3 was spiked as internal standard. Error bars represent 95% confidence levels.

The peaks of BPDE-DNA fluorescence were normalized with respect to Tb(NO3)3 internal standard. The standard curve for BPDE-DNA adducts generated from the data is shown in Figure 3. Similarly, the standard curve for BPDE-DNA adducts at the high concentration range is shown in Figure 4.

The limit of quantification for BPDE-DNA adducts was 3.3 fmol, with a signal-to-noise ratio of 46. This level of sensitivity corresponded to 6.4/10^10 nucleotides or 770/cell when using 22.5 µg of DNA, which was sufficient for analysis of BPDE-DNA adduct levels in cells during long-term low-dose exposure.

machinery. When plotted in log scale, the adduct level decreased linearly with time, following the first-order kinetics described by the following equations:

\[
\frac{dD(t)}{dt} = -kD(t)
\]

\[
D(t) = D_0 e^{-kt}
\]

\[
\ln D(t) = -kt + \ln D_0
\]

\[
T_{1/2} = \frac{\ln 2}{k}
\]

where \(D_0\) and \(D(t)\) are the levels of DNA adduct at time 0 and \(t\), respectively. \(K\) is the DNA adduct repair rate constant. \(T_{1/2}\) is the half-life for the repair of adducts.

The calculated DNA repair rate constants were 0.0532 and 0.0565 for AHH-1 and TK6 cells, respectively. Thus, the BPDE–DNA adduct half-lives were calculated to be 13.0 and 12.3 h for AHH-1 and TK6 cells, respectively, indicating similar capacities for repairing BPDE–DNA adducts. The rates of BPDE adduct repair in both cell lines were constant over the range of time studied, 36 h after the initial treatment. About 65% of DNA adducts were removed in 24 h in AHH-1 cells.

**Discussion**

**Improving Cryogenic Laser-Induced Fluorescence Spectroscopy FNLS To Quantify BPDE Adducts.** The DNA adducts of BPDE are the most frequently studied biomarkers of environmental exposure to B[a]P in cooked foods and air pollutants. These pyrenoid adducts have been well characterized and a battery of analytical methods have been employed for their detection and quantification (30–32). Previous studies indicate that total PAH–DNA and BaP–DNA adduct levels in human white blood cells are in the range of 0.2–3.9/10^8 nucleotides (or 24–468 adducts/cell) for controls and 0.4–19.5/10^8 nucleotides (or 48–2340 adducts/cell) for occupationally exposed individuals (33, 34).

Cryogenic laser-induced fluorescence spectroscopy is a highly sensitive and specific method for the analysis of BPDE–DNA adducts (8). Previous studies using this methodology attained a limit of quantification corresponding to 5000/10^8 nucleotides or 600,000/cell using 100 \(\mu\)g of DNA (18). To further increase sensitivity of cryogenic LIF, we sought to reduce background fluorescence caused by impurities and contamination. First, we used fused silica sample tubes that contained a low level of impurities (~1 ppm) that might contribute to background fluorescence when excited with ultraviolet light. We further tested various antifreeze agents. At an excitation wavelength of 346 nm, the ideal anti-freezing solvent should give minimal fluorescence particularly in the region of fluorescence peaks from test compounds. However, even most high-grade solvents contain trace amounts of impurities. We selected EG as the anti-freezing solvent instead of the more commonly used glycerol because of its higher purity, lower viscosity, and excellent anti-freezing properties. UV absorption data demonstrated that commercially available EG preparations contained fewer impurities compared to glycerol. Furthermore, EG has relatively lower viscosity, which facilitates its purification by column chromatography and reduces volumetric errors during sample uptake and mixing. EG used in this study was further purified by C18 column chromatography. DNA solutions can be mixed with EG to give 50% (by volume) mixture of EG/water, which allows for uniform and crystal-free freezing of genomic DNA solutions (0.75 mg/mL) of 10–50 kb length at cryogenic temperatures as low as 120 K. The incorporation of purified EG as the anti-freezing agent decreased the background, improved sensitivity and accuracy, and thus represented one of the major methodological improvements in BPDE–DNA adduct detection.

Internal standards are commonly used to correct for differences in light scattering and variations in instrumental factors among experiments. Dyes such as disodium fluorescein (18) and PAH molecules such as benzo[ghi]perylene sulfide (BDS) are widely used as internal standards for LIF analysis. However, these compounds either have fluorescence spectra that overlap with those of the pyrenoid adduct fluorescence peaks or have much shorter lifetimes compared to pyrenoid adducts. The present study is the first to demonstrate the value of using lanthanide salts as an internal standard in fluorescence assays. Among various lanthanide salts, terbium(III) nitrate pentahydrate Tb(NO₃)₃ (Sigma) was chosen because of its nonradioactivity, high solubility in water, ethanol, and ethylene glycol. Moreover, Tb(NO₃)₃ and BPDE–DNA have very similar fluorescence lifetimes but well-separated fluorescence spectra. The utilization of Tb(NO₃)₃ as internal standard greatly increased the sensitivity for detecting BPDE–DNA adducts. The limit of quantification for BPDE–DNA adducts reached 3.3 fmol (20.7 x 10⁹) from 22.5 \(\mu\)g of genomic DNA with a signal-to-noise ratio of 46, which corresponds to 6.4/10^8 nucleotides or 776/cell (Figure 2). Thus, the detection
limit using 22.5 µg of DNA was estimated to be around 0.2 fmol, 0.4/10⁹ nucleotides, or 50/cell based on signal to noise ration of three. The limit of detection and quantification can be further improved severalfold by loading larger quantities of DNA.

Other assays for low-level DNA adduct measurement include immunonassays (35, 36), ³²P-postlabeling (37–39), HPLC coupled with mass spectrometer (40–43), and HPLC with fluorescence detection (44, 45). These methodologies have adequate sensitivities to detect adducts in genomic DNA from human tissue. Despite the inability to detailed structural information, the FNLS method described in this study has several advantages over existing methodologies. For instance, immunoassays require the growth of specific antibodies for each carcinogen to be determined, necessitate relatively large quantities of DNA (200–300 µg/assay), and may have reduced specificity due to antibody cross-reactivity. Moreover, both immunoassays and fluorescence spectroscopy were developed for the analysis of targeted adducts and will typically provide no information for unknowns. ³²P-Postlabeling involves working with large quantities of harmful radioisotopes and identification of unknown adducts remains challenging. While providing detailed structural information, HPLC-mass spectrometry based assays require larger amount of DNA, and digestion of DNA to nucleosides together with subsequent enrichment and clean up. Although our enhanced FNLS method cannot provide detailed structural information for each adduct (BPDE-da, BPDE-dg, and various diastereomeric forms), it has the advantages of being an inexpensive, rapid, robust, highly sensitive technique for quantifying total BPDE – DNA adduct levels in genomic DNA. Moreover, the method can be used to quantify the level of total adducts derived from dibenzo(a,l)pyrene diol epoxides, malonaldehyde, etc.

DNA Adduct Levels in Human Lymphoblastoid Cells after BPDE Treatment. It has been reported that BPDE binds to hydrophobic interiors of carrier proteins such as serum albumin in aqueous solution (46). The culture medium in this experiment contained 5% of horse serum. Such noncovalent interactions can prolong the half-life of BPDE from a few minutes in aqueous solution to a few hours in the presence of serum albumin, and provides a mechanism of transport from medium to target macromolecules within the cell. BPDE can then react with other macromolecules such as DNA. Therefore, the kinetics for BPDE – DNA adduction formation in cultured human cells after treatment is expected to be instantaneous, followed by a persistent but lower rate of adduction formation while serum stabilized mutagen is in equilibrium with the unbound form with simultaneous and continuous adduct removal. As the rate of adduction formation declined, the adduct removal rate became exponential. Both AHH-1 and TK6 cells were shown to have first-order DNA repair kinetics with very similar repair rates (Figures 5 and 6). Using a variety of methods, including synchronous fluorescence spectrophotometry (47), immunoassay (48, 49), and ³²P-postlabeling (50), investigators have also observed exponential BPDE – DNA adduct removal in different murine tissues in vivo and in vitro.

Conclusion

A highly sensitive, economical and rapid method using FNLS was used for monitoring the level of total BPDE – DNA adducts formed in human cells upon treatment. For example, it was possible to monitor BPDE – DNA adduct levels in treated cells as a function of time using intact genomic DNA, without degrading the DNA to nucleoside level. The limit of quantification for BPDE – DNA adducts was 2 × 10⁹, or 3.3 fmol of adducts, which corresponded to 6.4 adducts/10⁹ nucleotides or 776 adducts/cell when using 22.5 µg of DNA. Thus, the level of sensitivity attained by our assay was comparable to that of ³²P-postlabeling and HPLC coupled with mass spectrometry and was sufficient for analysis of BPDE – DNA adduct levels in cells during long-term low-dose exposure experiments that mimic most human environmental exposure to mutagens.

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References


Quantifying BPDE – DNA Adducts Using Fluorescence

Modulation of benzo[a]pyrene diol-epoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 21, 35–41.


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