Free Radical Biology & Medicine 48 (2010) 318–324

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Benzene and dopamine catechol quinones could initiate cancer or neurogenic disease

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ARTICLE INFO

Article history:
Received 31 August 2009
Revised 22 October 2009
Accepted 4 November 2009
Available online 10 November 2009

Keywords:
1,4-Michael addition
Catechol quinones
Depurinating DNA adducts
Kinetics of depurination

ABSTRACT

Catechol quinones of estrogens react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts. Loss of these adducts from DNA creates apurinic sites that can generate mutations leading to cancer initiation. We compared the reactions of the catechol quinones of the leukemogenic benzene (CAT-Q) and *N*-acetyldopamine (NADA-Q) with 2′-deoxyguanosine (dG) or DNA. NADA was used to prevent intramolecular cyclization of dopamine quinone. Reaction of CAT-Q or NADA-Q with dG at pH 4 afforded CAT-4-N7dG or NADA-6-N7dG, which lost deoxyribose with a half-life of 3 h to form CAT-4-N7Gua or 4 h to form NADA-6-N7Gua. When CAT-Q or NADA-Q was reacted with DNA, N3Ade adducts were formed and lost from DNA instantaneously, whereas N7Gua adducts were lost over several hours. The maximum yield of adducts in the reaction of CAT-Q or NADA-Q with DNA at pH 4 to 7 was at pH 4. When tyrosinase-activated CAT or NADA was reacted with DNA at pH 5 to 8, adduct levels were much higher (10- to 15-fold), and the highest yield was at pH 5. Reaction of catechol quinones of natural and synthetic estrogens, benzene, naphthalene, and dopamine with DNA to form depurinating adducts is a common feature that may lead to initiation of cancer or neurodegenerative disease.

Experiments on estrogen metabolism, formation of DNA adducts, mutagenicity, cell transformation, and carcinogenicity led to and support the hypothesis that reaction of specific metabolites, mostly catechol estrogen-3,4-quinones, with DNA forms small amounts of stable adducts (<1%) and predominant amounts of depurinating adducts (>99%) [1,2]. This reaction occurs by a proton-assisted 1,4-Michael addition [3]. The depurinating adducts obtained are 4-hydroxyestrone(estradiol)-1-N3Ade [4-OHE$_1$(E$_2$)-1-N3Ade][1] and 4-OHE$_1$(E$_2$)-1-N7Gua. The stable adducts remain in DNA unless removed by repair, whereas the depurinating adducts detach from DNA, leaving behind apurinic sites. Errors in the repair of these sites can lead to the critical mutations initiating breast, prostate, and other human cancers [1,2,4,5].

Strong evidence has also been obtained that the synthetic estrogen hexestrol and the human carcinogen diethylstilbestrol are metabolized predominantly to their respective catechols. When the catechols are oxidized to quinones, they also react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts that are analogous to those formed by the catechol estrogen-3,4-quinones [6–8]. Similarly, the catechol quinone of naphthalene reacts with DNA in vitro and in vivo to form analogous depurinating N3Ade and N7Gua adducts [9,10].

We have evidence that the *ortho*-quinones of the leukemogenic benzene and the neurotransmitter dopamine can also react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts [11] analogous to those formed by the natural and synthetic estrogens, as well as naphthalene. In this article, we report a further study of the benzene catechol (1,2-dihydroxybenzene; CAT) and *N*-acetyldopamine (NADA), which is itself a catechol (Fig. 1). Benzene is metabolized to phenol in the liver by cytochrome P450 2E1 [12,13]. Other metabolites include CAT and hydroquinone (1,4-dihydroxybenzene) [14]. Oxidation of CAT and hydroquinone is catalyzed by peroxidases, forming quinones that can exert myelotoxic effects [15] and produce stable [16,17] and depurinating [11] DNA adducts. In fact, higher levels of peroxidases and the lack of quinone reductase in the bone marrow allow formation of toxic quinones without the possibility of their being reduced [18].

One of the major metabolic pathways of the neurotransmitter dopamine is oxidation to its catechol quinone, which at neutral pH regularly undergoes intramolecular cyclization by 1,4-Michael addition, followed by oxidation to form leukochrome and then aminochrome. Polymerization of the aminochrome leads to neuromelanin (Fig. 2, top). At lower pH, however, partial protonation of the amino group of dopamine slows down the intramolecular cyclization of the dopamine quinone, rendering competitive the reaction of the quinone with DNA to form depurinating N3Ade and N7Gua adducts (Fig. 2, bottom). Although the two depurinating adducts were obtained from dopamine activated by tyrosinase [11], NADA was used in this study

Abbreviations: Ade, adenine; CAT, 1,2-dihydroxybenzene; CAT-Q, catechol-1,2-quinone; DA, dopamine; dG, 2′-deoxyguanosine; DMF, dimethylformamide; ESI, electrospray ionization; Gua, guanine; NADA, *N*-acetyldopamine; NADA-Q, *N*-acetyldopamine-3,4-quinone; OHE$_1$(E$_2$), hydroxyestrone(estradiol); TFA, trifluoroacetic acid; UPLC-MS/MS, ultraperformance liquid chromatography/tandem mass spectrometry.

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doi:10.1016/j.freeradbiomed.2009.11.002

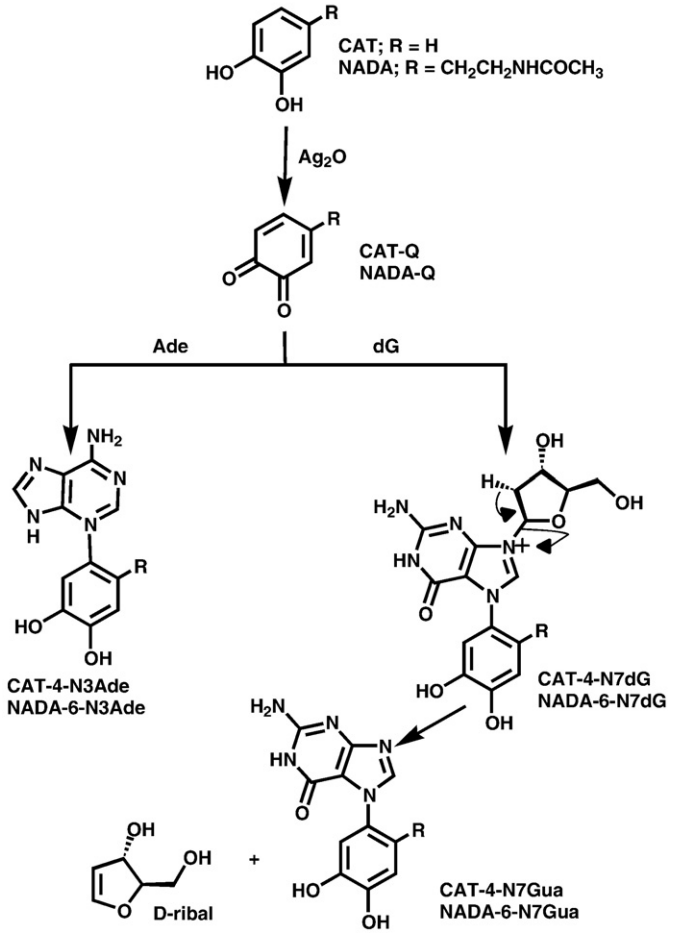

Fig. 1. Oxidation CAT and NADA to their respective quinones and reaction of the quinones with Ade or dG to form N3Ade and N7Gua adducts.

to protect the amino group of dopamine and totally avoid the pathway of intramolecular cyclization after formation of the quinone. In this way we could compare the ability of the catechol quinone of benzene and the dopamine quinone to react intermolecularly via 1,4-Michael addition at various pH.

Materials and methods

Chemicals and reagents

CAT, Ag_2O, potassium tetraborate, acetic anhydride, formic acid, acetic acid, trifluoroacetic acid (TFA), dimethylformamide (DMF), and dimethyl sulfoxide-d_6 were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as such without further purification. 2′-Deoxyguanosine (dG) and calf thymus DNA were purchased from USB (Cleveland, OH, USA). Dopamine (DA) hydrochloride and mushroom tyrosinase were purchased from Sigma Chemical (St. Louis, MO, USA). The standard depurinating adducts of CAT, i.e., CAT-4-N7Gua and CAT-4-N3Ade, and those of NADA, NADA-6-N7Gua and NADA-6-N3Ade, were synthesized according to the procedure reported earlier [11].

Instrumentation

UV

UV spectra were obtained during HPLC by using a Waters (Milford, MA, USA) 996 or 2996 photodiode array detector for all compounds synthesized. HPLC separations were monitored at 290 nm for both CAT and NADA.

HPLC

Analytical HPLC was conducted on a Waters 2690 separations module equipped with a Waters 996 or 2996 photodiode array detector and a reverse-phase Phenomenex Luna-2 C-18 column (250 × 4.6 mm, 5 μm; 120 Å; Torrance, CA, USA). For CAT, the column was eluted starting with 8% CH_3CN in H_2O (0.25% TFA) and continuing

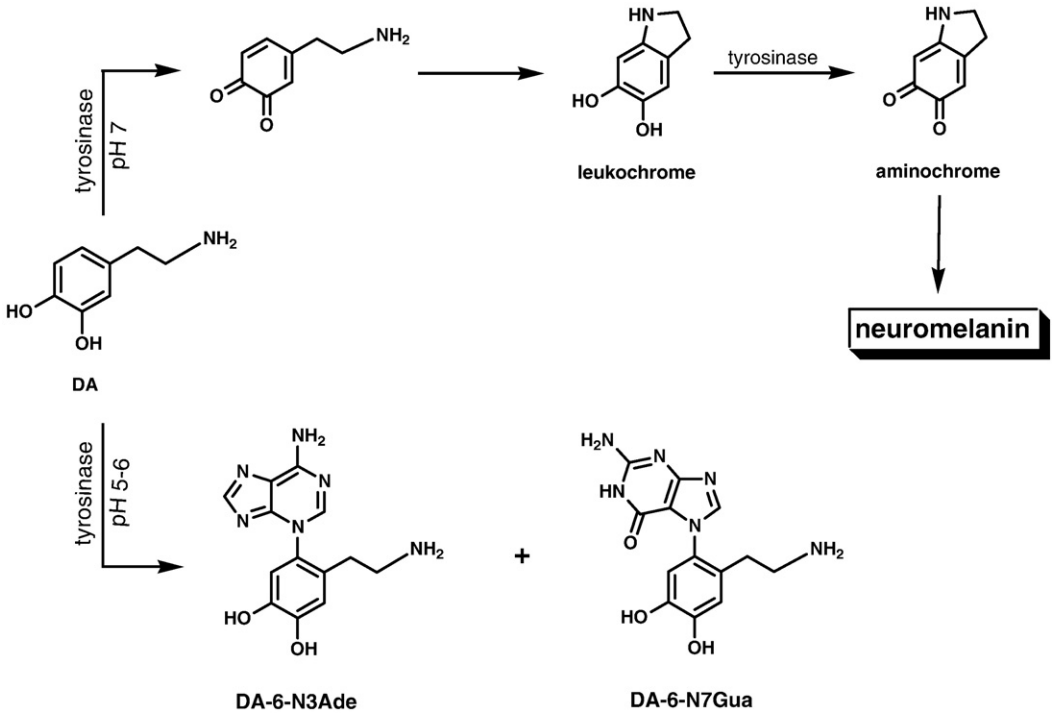

Fig. 2. Metabolism of dopamine to form neuromelanin and depurinating DNA adducts.

with a linear gradient to 10% CH_3CN in 25 min. For NADA, the column was eluted starting with 6% CH_3CN in H_2O (0.25% TFA) and then with a linear gradient to 8% CH_3CN in 20 min at a flow rate of 1 ml/min.

Mass spectrometry

Electrospray ionization (ESI)-MS. All experiments were performed on a Waters QuattroMicro triple-quadrupole mass spectrometer by using ESI in the positive ion mode, with an ESI-MS capillary voltage of 3.0 kV, an extractor cone voltage of 2 V, and a detector voltage of 650 V. Desolvation gas flow was maintained at 400 l/h. Cone gas flow was set at 60 l/h. Desolvation temperature and source temperature were set to 200 and 100°C, respectively. The acquisition range was 50–700 Da. The sample was introduced to the source at a flow rate of 10 μl/min by using a built-in pump.

UPLC-MS/MS. Samples were analyzed on an Acquity UPLC system attached to a MicroMass QuattroMicro triple-quadrupole mass spectrometer. The 10-μl injections were made to a Waters Acquity UPLC $BEHC_{18}$ column (1.7 μm, 1×100 mm). The instrument was operated in the positive ESI mode. All aspects of system operation, data acquisition, and processing were controlled using QuanLynx version 4.0 software (Waters). The column was eluted with 5% CH_3CN in H_2O (0.1% formic acid) for 10 min at a flow rate of 150 μl/min. Ionization was achieved using the following settings: capillary voltage 3 kV, cone voltage 15–60 V, source block temperature 100°C, desolvation temperature 200°C, with a nitrogen flow of 400 l/h. Nitrogen was used as both the desolvation and the auxiliary gas. Argon was used as the collision gas. Three-point calibration curves covering the range of analytes detected were run for each standard.

Synthesis of NADA

NADA was synthesized from DA with a slight modification of the reported method [19]. DA hydrochloride (0.8 g) was dissolved in 40 ml of 10% potassium tetraborate that had been purged with nitrogen. Then 0.44 ml of 99% acetic anhydride was slowly added over a period of 30 min to the DA hydrochloride solution under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 8 h, and then 3 ml of concentrated formic acid was added to precipitate boric acid. After filtration, the supernatant was extracted with ethyl acetate (3×50 ml). The combined organic layers were concentrated and purified by column chromatography on silica gel using $CHCl_3$:CH_3OH (1:1) as the solvent system. Fractions containing common thin-layer chromatography spots were pooled and concentrated to obtain pure NADA (540 mg, 66%). The NMR and MS data for the compound matched exactly those already reported in the literature [19].

Reaction of CAT-Q or NADA-Q with dG and loss of deoxyribose

CAT (11 mg, 0.1 mmol) or NADA (20 mg, 0.1 mmol) was dissolved in 2 ml of DMF and stirred at 0°C. To this solution was added Ag_2O (140 mg, 0.6 mmol) portion-wise and the reaction mixture was stirred at 0 °C for 30 min. A dark-colored solution of CAT-Q or NADA-Q was filtered quickly through a syringe filter into a solution of dG (142 mg, 0.5 mmol) in H_2O:CH_3COOH (2 ml, 1:1) and the pH of the solution was adjusted to 4. The reaction mixture was stirred at room temperature and aliquots at various time intervals (0.33, 0.66, 1, 2, 3, 4, 5, 7, 8, 12, 16, 20, and 24 h) were analyzed by using analytical HPLC monitored at 290 nm. The CAT-4-N7dG and CAT-4-N7Gua peaks were identified and showed R_t at 9.5 and 10.2 min, respectively. The NADA-6-N7dG and NADA-6-N7Gua peaks were eluted at 8.8 and 10.4 min, respectively. The compounds were analyzed and quantified at 290 nm using Empower software after comparing the response of samples with the calibration curves made with the standard depurinating adduct, i.e., CAT-4-N7Gua and NADA-6-N7Gua. The rationale for using the N7Gua adducts for quantitation of the N7dG adducts derives from their similar UV spectra. The reactions were conducted and analyzed in triplicate.

Kinetics of depurinating adduct formation after reaction of DNA with CAT-Q or NADA-Q

CAT (19.2 mg, 174 μmol) or NADA (33.9 mg, 174 μmol) was dissolved in 1 ml of acetone and stirred at 0 °C. Then Ag_2O (242.2 mg, 6 equivalents) was slowly added. After 30 min, the solution was filtered through a Gelman acrodisc. Five microliters of the freshly prepared quinone (8.7 μmol of CAT-Q or NADA-Q) in acetone was reacted with 3 mM DNA in a 10-ml reaction mixture at pH 4. At various time points (1, 2, 3, 4, 5, 6, 7, and 10 h), aliquots of the mixtures were treated quickly with 2 volumes of ethanol to remove DNA and the supernatants were analyzed for the formation of depurinating adducts, as described above. The reactions were performed in triplicate and control experiments were conducted with 5 μl of acetone in place of the quinone solution.

Covalent binding of CAT-Q or NADA-Q to DNA at various pH

Five microliters (87 μM final concentration) of a stock solution of CAT-Q or NADA-Q was mixed with DNA in a total 10-ml reaction mixture containing 3 mM calf thymus DNA in 0.1 M sodium acetate (for pH 4 or 5) or 0.1 M sodium potassium phosphate (pH 6 or 7). After incubation at 37 °C for 10 h, the DNA was precipitated with 2 volumes of ethanol; the supernatant was evaporated and dissolved in methanol (1 ml), lyophilized, and redissolved in 50 μl of methanol: water (1:1). The final solution was passed through a 5000 MW cut-off filter (Millipore, Billerica, MA, USA) and 10 μl of each sample was analyzed for depurinating adducts by UPLC-MS/MS. Control reactions were carried out under identical conditions using 50 μl of acetone in place of the quinone solution.

Covalent binding of tyrosinase-activated CAT or NADA to DNA at various pH

Stock solutions of CAT (0.96 mg) or NADA (1.69 mg) were made in 50 μl of acetone and 5 μl of each solution was mixed with DNA in a total 10-ml reaction mixture containing 3 mM calf thymus DNA in 0.1 M sodium acetate (pH 5) or 0.1 M sodium potassium phosphate (pH 6, 7, or 8) and 1 mg of mushroom tyrosinase (2577 units). The reaction mixture was incubated at 37°C in the presence of air for 10 h. After precipitation of DNA with ethanol, the supernatant was used for analysis of depurinating adducts, as described above. Control reactions were carried out under identical conditions using 50 μl of acetone in place of the CAT or NADA solution.

Results

Formation of CAT-4-N7dG and NADA-6-N7dG adducts

The reaction of CAT-Q with dG at pH 4.0 (Fig. 1) afforded initially a labile adduct as observed by HPLC analysis (retention time 9.5 min, data not shown). After 1 h an aliquot of the reaction mixture was directly infused into the mass spectrometer (Fig. 3) to afford the molecular ions $[M]^+$ at m/z 376, 267, 260, and 152, corresponding to CAT-4-N7dG ($C_{16}H_{18}N_5O_6^+$), dG, CAT-4-N7Gua, and Gua, respectively. MS/MS analysis of the peak at m/z 376 afforded a daughter ion at m/z 260, corresponding to CAT-4-N7Gua. Attempts to isolate and record the NMR spectrum of the CAT-4-N7dG adduct were not successful, because the compound decomposed during the removal of the solvent.

Analogously, the reaction of NADA-Q with dG at pH 4.0 yielded initially a labile adduct, NADA-6-N7dG, which slowly converted to

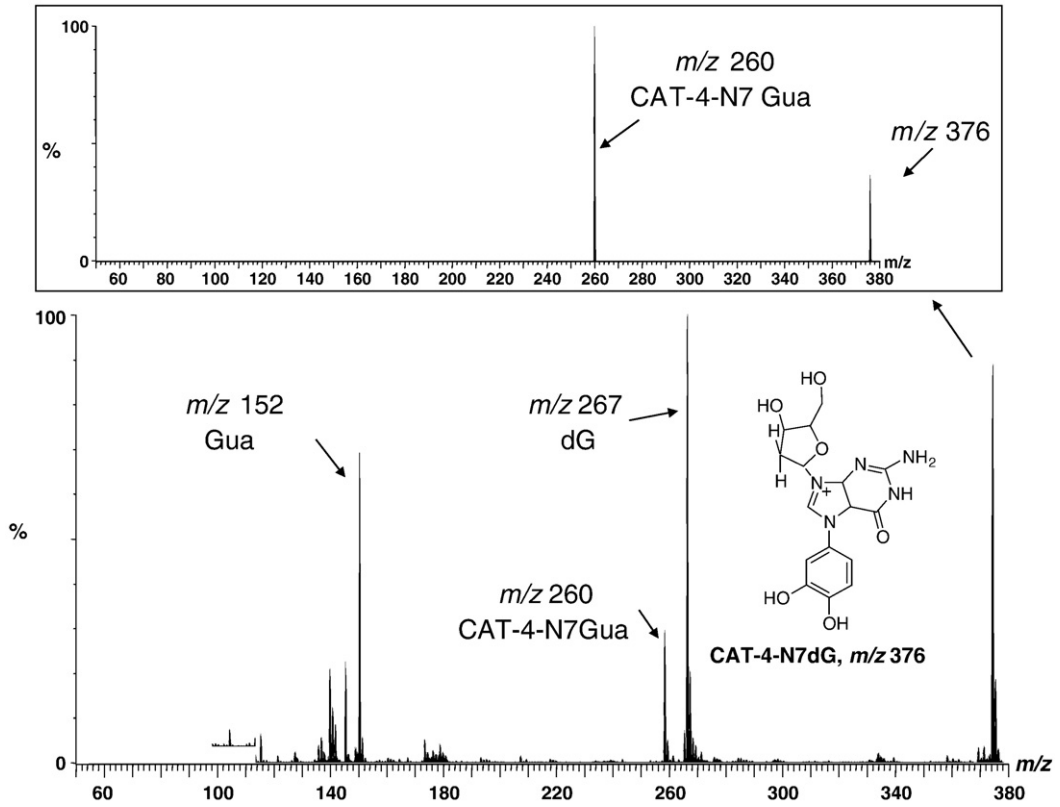

Fig. 3. Mass spectrum of the reaction mixture of CAT-Q with dG after 1 h at 22 °C. Top: MS/MS of m/z 376, CAT-4-N7dG, showing the daughter ion at m/z 260, CAT-4-N7Gua.

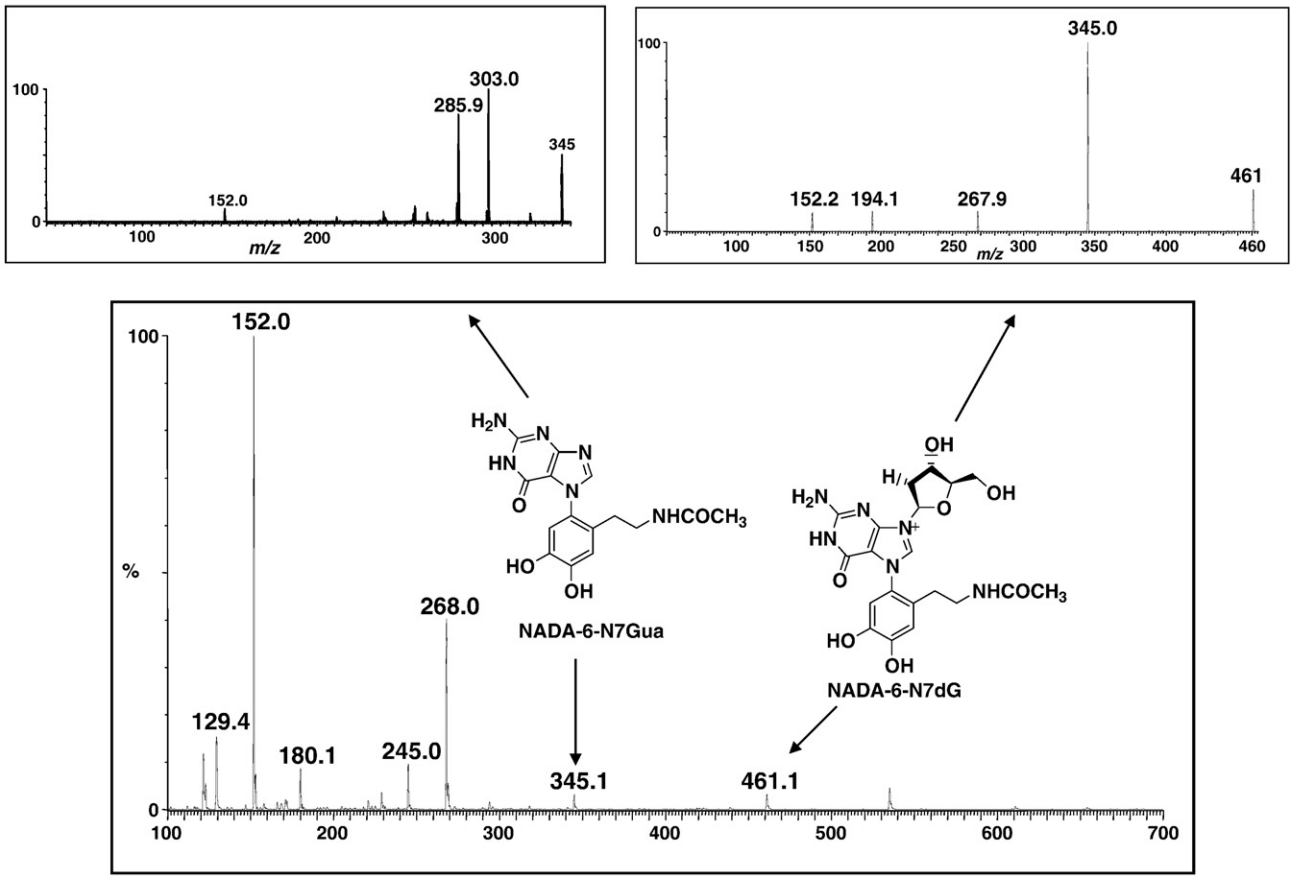

Fig. 4. Mass spectrum of the reaction mixture of NADA-Q with dG after 3 h at 22 °C. Top: Right, MS/MS of m/z 461, NADA-6-N7dG, showing the daughter ions at m/z 345, NADA-6-N7Gua, and m/z 152, Gua. Left, MS/MS of m/z 345, NADA-6-N7Gua, showing the daughter ions at m/z 303, NADA-6-N7Gua minus the acetyl group, and m/z 152, Gua.

the depurinating NADA-6-N7Gua adduct. The reaction mixture was analyzed after 3 h by direct infusion into the mass spectrometer. A molecular ion $[M]^+$ peak at $m/z = 461$ (Fig. 4) showed the formation of NADA-6-N7dG adduct that was selectively fragmented to its daughter ion at m/z 345, corresponding to the depurinating NADA-6-N7Gua adduct; m/z 303, corresponding to NADA-6-N7Gua minus an acetyl group; and m/z 152, corresponding to the released Gua moiety.

Kinetics of loss of the deoxyribose moiety

Loss of deoxyribose from CAT-4-N7dG

The rate of loss of the deoxyribose moiety was studied by reacting CAT-Q with dG and analyzing aliquots of the reaction mixture at various time points. The time course of this reaction is represented in Fig. 5 by plotting the concentrations of CAT-4-N7dG and CAT-4-N7Gua over time. The maximum formation (6.4 ± 1.1 mM) of the CAT-4-N7dG adduct was obtained within 20 min, indicating that most of the CAT-Q had reacted during this time. This adduct subsequently released the deoxyribose moiety and was converted to CAT-4-N7Gua. The concentration of CAT-4-N7Gua was minimal (1.6 ± 0.3 mM) at 20 min, but slowly increased with time, indicating the slow depurination of CAT-4-N7dG. The concentrations of the CAT-4-N7dG and CAT-4-N7Gua were almost equal (4.4 ± 0.6 and 4.3 ± 0.8 mM, respectively) at 2 h. By 12 h, almost complete conversion of CAT-4-N7dG to CAT-4-N7Gua (9.5 ± 1.4 mM) was observed, and after that the amount of the depurinating adduct remained constant. The half-life of CAT-4-N7dG conversion to CAT-4-N7Gua was approximately 3 h (Fig. 5).

Loss of deoxyribose from NADA-6-N7dG

In the reaction between NADA-Q and dG, the concentration of NADA-Q decreased rapidly, with the concomitant formation of NADA-6-N7dG. This adduct subsequently released the deoxyribose moiety and was converted to the depurinating NADA-6-N7Gua adduct. The concentration of the N7dG adduct increased initially and reached its highest level of 1.8 ± 0.6 mM at 2 h and then decreased continuously until it reached a trace amount of <0.1 mM at 12 h (Fig. 6). On the other hand, the amount of the N7Gua adduct was minimal (0.2 ± 0.2 mM) at 30 min and then increased continuously until it reached the maximum amount of 2.9 ± 0.7 mM at 12 h. After 12 h, the adduct concentrations remained constant as shown in Fig. 6. The half-life of NADA-6-N7dG conversion to NADA-6-N7Gua was approximately 4 h (Fig. 6).

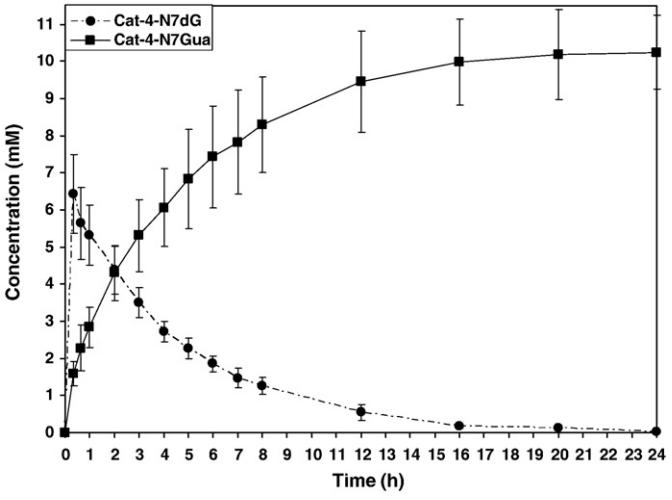

Fig. 5. Time course of the reaction of CAT-Q with dG to form CAT-4-N7dG and its conversion to CAT-4-N7Gua. CAT (0.05 M) was oxidized to CAT-Q with 3 M Ag_2O in 2 ml of DMF and filtered into 0.25 M dG in 2 ml of H_2O:CH_3COOH (1:1). The pH was adjusted to 4 and the reaction continued at room temperature.

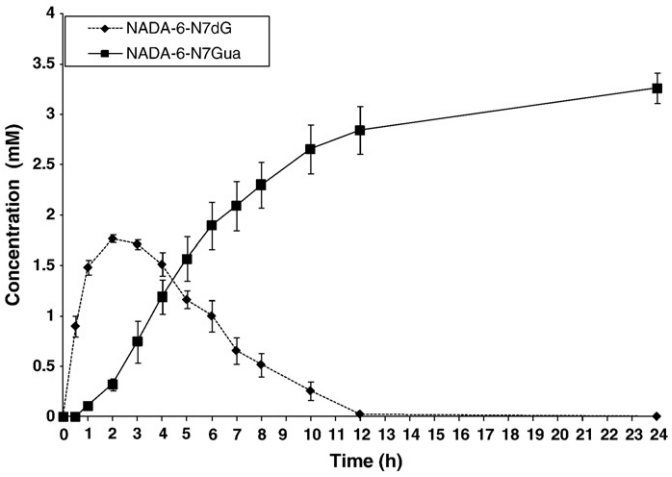

Fig. 6. Time course of the reaction of NADA-Q with dG to form NADA-6-N7dG and its conversion to NADA-6-N7Gua. The same reaction conditions as for Fig. 5 were used, except that NADA was used instead of CAT.

Time course of the formation of depurinating adducts of CAT and NADA in DNA

After finding the optimum conditions for the formation of depurinating adducts, we conducted a time course study by reacting CAT-Q or NADA-Q with DNA at pH 4. Aliquots of the reaction mixture were analyzed at various time points. As shown in Fig. 7, depurination of the N3Ade adduct (26.0 ± 4.4 μmol/mol DNA-P) was almost complete within 1 h of incubation time and its level remained constant after that. On the other hand, depurination of the N7Gua adduct was not instantaneous. A gradual increase in the level of the N7Gua adduct formed was observed, starting with 3.4 ± 0.1 μmol/mol DNA-P at 1 h and reaching a maximum level of 12.1 ± 1.4 μmol/mol DNA-P at about 6 h.

In the reaction between NADA-Q and DNA, instantaneous depurination of the N3Ade adduct was observed and its level of 14.8 ± 0.7 μmol/mol DNA-P remained unchanged after 1 h (Fig. 8). Depurination of the N7Gua adduct was slow. At 1 h, a minimal amount, 1.2 ± 0.1 μmol/mol DNA-P, of the N7Gua adduct was observed in the reaction mixture, which continuously increased until it reached its maximum level of 15.2 ± 1.2 μmol/mol DNA-P at 5 h. The adduct level remained unchanged afterward (Fig. 8).

The half-life of CAT-4-N7dG and NADA-6-N7dG in DNA was approximately 3 h.

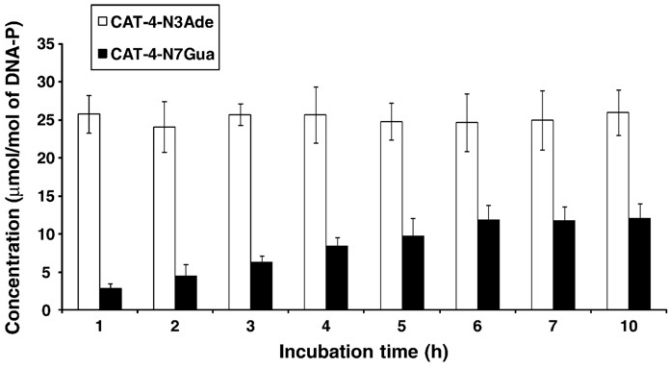

Fig. 7. Time course of the formation of CAT-4-N3Ade and CAT-4-N7Gua after the reaction of CAT-Q with DNA at pH 4. CAT-Q (87 μM) was reacted with 3 mM DNA at pH 4 and room temperature.

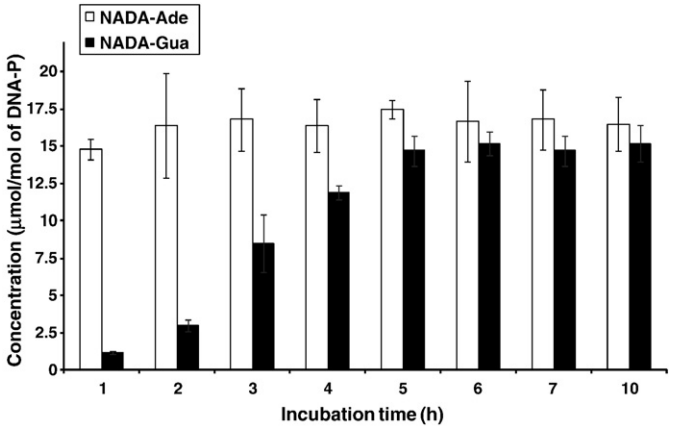

Fig. 8. Time course of the formation of NADA-6-N3Ade and NADA-6-N7Gua after the reaction of NADA-Q with DNA at pH 4. The same reaction conditions as for Fig. 7 were used, except that NADA-Q was used instead of CAT-Q.

Formation of depurinating adducts of CAT and NADA at various pH

The effect of pH on the formation of depurinating adducts was studied by reacting CAT-Q (or NADA-Q) or enzymatically activated CAT (or NADA) with DNA. The level of the depurinating adducts was measured by using UPLC-MS/MS. For CAT, the highest level (29.0 ± 4.5 μmol/mol DNA-P for N3Ade and 15.4 ± 2.6 μmol/mol DNA for N7Gua) of depurinating adducts was detected at pH 4 (Fig. 9A). When the pH of the reaction mixture was increased to 5, the level of the N3Ade adduct was reduced to 10.1 ± 2.1 μmol/mol DNA-P and that of the N7Gua adduct to 3.3 ± 0.3 μmol/mol DNA-P. At pH 6, the level of CAT-4-N3Ade was 3 ± 0.5 μmol/mol DNA-P, and CAT-4-N7Gua, 1.5 ± 0.4 μmol/mol DNA-P. At neutral pH, a sharp reduction in the level of depurinating adducts was observed, giving a level of 1.1 ± 0.3 μmol/mol DNA-P for the N3Ade adduct and 0.4 ± 0.1 μmol/mol DNA-P for N7Gua. Quite surprisingly, the N3Ade adduct was formed in much higher amounts than the corresponding N7Gua adduct (Fig. 9A). When tyrosinase-activated CAT was reacted with DNA at various pH (5–8, Fig. 9B), the highest level of depurinating adducts was observed at pH 5, 95 ± 15 μmol/mol DNA-P for N3Ade and 114 ± 18 μmol/mol DNA-P for N7Gua. Again, at higher pH (6–8), the depurinating adduct levels were reduced. However, slightly more Gua than Ade adducts were detected at pH 5 and 6, which is reversed for pH 7 and 8.

For NADA, the reaction between NADA-Q and DNA at pH 4 was optimum with respect to the formation of depurinating adducts (NADA-6-N7Gua and NADA-6-N3Ade, Fig. 10A). The N3Ade and N7Gua adducts were detected in similar amounts, 16.4 ± 3.2 and 13.8 ± 2.7 μmol/mol DNA-P, respectively. By increasing the pH to 5, the amounts of the depurinating adducts were lowered to half, with the formation of 7.2 ± 0.4 μmol/mol DNA-P for the N3Ade adduct and 7.7 ± 0.3 μmol/mol DNA-P for the N7Gua adduct. At neutral pH, the amount of the N3Ade adduct was 2.0 ± 0.3 μmol/mol DNA-P and that of the N7Gua adduct was 1.8 ± 0.3 μmol/mol DNA-P. A similar trend was seen when NADA was activated with tyrosinase in the presence of DNA and the reaction was conducted at various pH (5–8, Fig. 10B).

Discussion

Reaction of catechol quinones with nucleophiles occurs via a proton-assisted 1,4-Michael addition [3]. In this article, we have

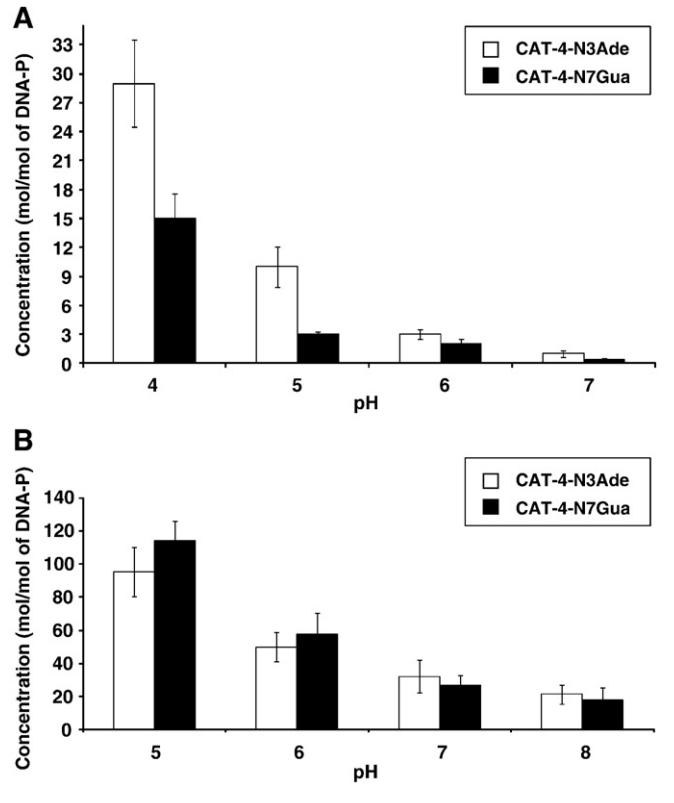

Fig. 9. Effect of pH on formation of the depurinating CAT-4-N3Ade and CAT-4-N7Gua adducts by reaction of (A) CAT-Q with DNA for 10 h and (B) tyrosinase-activated CAT with DNA for 10 h. (A) CAT-Q (87 μM) was reacted with 3 mM DNA at the indicated pH and 37 °C or (B) 87 μM CAT was reacted with 3 mM DNA in the presence of 2577 units of mushroom tyrosinase at the indicated pH and 37°C.

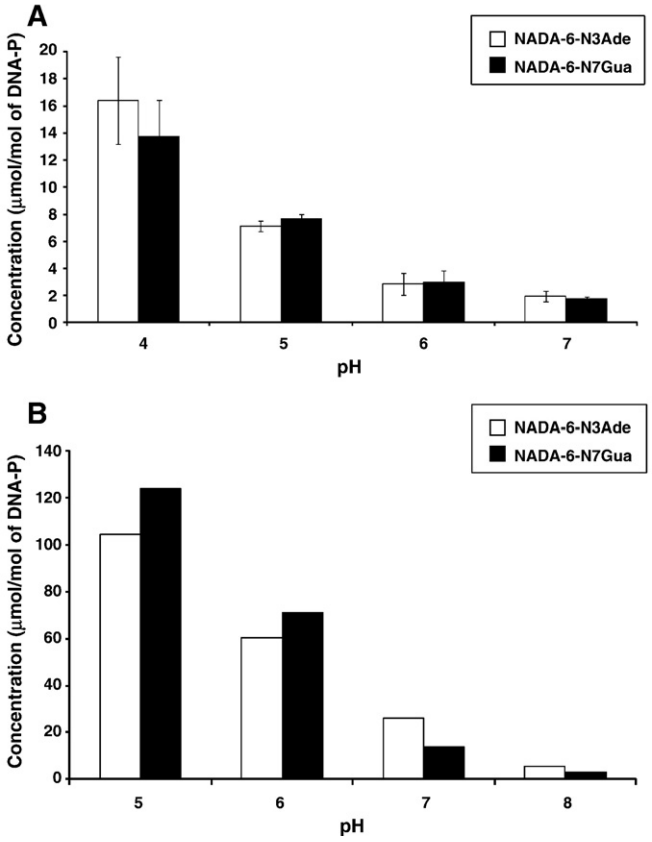

Fig. 10. Effect of pH on formation of the depurinating NADA-6-N3Ade and NADA-6-N7Gua adducts by reaction of (A) NADA-Q with DNA for 10 h and (B) tyrosinase-activated NADA with DNA for 10 h. The same reaction conditions as for Fig. 9 were used, except that (A) NADA-Q or (B) NADA was used instead of CAT-Q or CAT.

compared the reaction of the catechol quinone of benzene, CAT-Q, with dG or DNA to that of the catechol quinone of NADA, NADA-Q.

Reaction of the quinones with dG forms N7dG adducts that slowly lose the deoxyribose moiety to form N7Gua adducts. We have investigated the kinetics of this loss of the deoxyribose moiety for both CAT-4-N7dG and NADA-6-N7dG at pH 4 (Figs. 5 and 6) and observed the rate of disappearance of the N7dG adducts with formation of the N7Gua adducts. The conversion of the CAT-4-N7dG adduct to the N7Gua adduct had a half-life of approximately 3 h (Fig. 5), whereas the conversion of the NADA-6-N7dG adduct had a half-life of approximately 4 h (Fig. 6).

A further comparison of CAT-Q and NADA-Q was obtained by reaction of the quinones with DNA at pH 4. With DNA, it was observed that the CAT-4-N3Ade and NADA-6-N3Ade adducts were formed and lost from DNA instantaneously, whereas the N7Gua adducts were lost more slowly, with half-lives of approximately 3 h (Figs. 7 and 8). With CAT-Q, the amount of the N3Ade adducts was more than twice that of the N7Gua adduct after 10 h (Fig. 7). In contrast, NADA-Q formed almost equal amounts of the N3Ade and N7Gua adducts in 10 h (Fig. 8).

Finally, the formation of N3Ade and N7Gua adducts by reaction of CAT-Q with DNA was studied at pH 4 to 7 (Fig. 9A). The maximum yield of both adducts was observed at pH 4, whereas at pH 7 the yield was negligible. In contrast, when tyrosinase-activated CAT was reacted with DNA at pH 5 to 8 (Fig. 9B), the amounts of the N3Ade and N7Gua adducts were about the same. The highest yield was at pH 5 and the yield decreased as the pH increased. The amounts of the adducts were much larger than those obtained from the reaction of the CAT-Q with DNA. For example, at pH 5, the amounts of the adducts obtained from tyrosinase-activated CAT were at least 10 times higher than those obtained with CAT-Q (Figs. 9A and 9B). This is presumably due to the instability of the quinone and the efficient reaction of the catechol after intercalation in the DNA and activation to quinone by tyrosinase. This mechanism has been demonstrated with the catechol of diethylstilbestrol [8].

When formation of N3Ade and N7Gua adducts by reaction of NADA-Q with DNA was studied at pH 4 to 7 (Fig. 10A), the maximum yield of both adducts was observed at pH 4 and decreased as the pH rose from 5 to 7. Both adducts were observed at similar levels at all pH. When NADA was activated by tyrosinase to react with DNA at pH 5 to 8, the amounts of adducts were at least 15 times higher than after the direct reaction of NADA-Q with DNA (Fig. 10B). Again, this is presumably due to the instability of NADA-Q and the efficient reaction of NADA after intercalation into the DNA and activation to NADA-Q by tyrosinase. The levels of the N3Ade and N7Gua adducts were not significantly different from each other.

The catechol quinones of natural [20–22] and synthetic [6–8] estrogens, benzene [11], naphthalene [9,10], and dopamine [11] react with DNA by 1,4-Michael addition to form predominantly depurinating N3Ade and N7Gua adducts. With all of these compounds, the N3Ade adduct depurinates instantaneously from DNA, whereas the N7Gua adduct depurinates slowly, with a half-life of a few hours. These common features may lead to the initiation of cancer or neurodegenerative disease.

Acknowledgments

This research was supported by Grants P01 CA49210 from the National Cancer Institute and DAMD17-03-10229 from the Department of Defense Breast Cancer Research Program and by Prevention LLC. Core support at the Eppley Institute was provided by Grant P30 CA36727 from the National Cancer Institute.

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