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Slow loss of deoxyribose from the N7deoxyguanosine adducts of estradiol-3,4-quinone and hexestrol-3′,4′-quinone. Implications for mutagenic activity

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Abstract

A variety of evidence has been obtained that estrogens are weak tumor initiators. A major step in the multi-stage process leading to tumor initiation involves metabolic formation of 4-catechol estrogens from estradiol (E_2) and/or estrone and further oxidation of the catechol estrogens to the corresponding catechol estrogen quinones. The electrophilic catechol quinones react with DNA mostly at the N-3 of adenine (Ade) and N-7 of guanine (Gua) by 1,4-Michael addition to form depurinating adducts. The N3Ade adducts depurinate instantaneously, whereas the N7Gua adducts depurinate with a half-life of several hours. Only the apurinic sites generated in the DNA by the rapidly depurinating N3Ade adducts appear to produce mutations by error-prone repair. Analogously to the catechol estrogen-3,4-quinones, the synthetic nonsteroidal estrogen hexestrol-3′,4′-quinone (HES-3′,4′-Q) reacts with DNA at the N-3 of Ade and N-7 of Gua to form depurinating adducts. We report here an additional similarity between the natural estrogen E_2 and the synthetic estrogen HES, namely, the slow loss of deoxyribose from the N7deoxyguanosine (N7dG) adducts formed by reaction of E_2-3,4-Q or HES-3′,4′-Q with dG. The half-life of the loss of deoxyribose from the N7dG adducts to form the corresponding 4-OHE_2-1-N7Gua and 3′-OH-HES-6′-N7Gua is 6 or 8 h, respectively. The slow cleavage of this glycosyl bond in DNA seems to limit the ability of these adducts to induce mutations.

Keywords: Steroidal and nonsteroidal estrogens; Catechol quinones; 1,4-Michael addition; Depurinating DNA adducts

1. Introduction

The natural and synthetic estrogens have been found to be carcinogenic in animal model studies [1–5]. The nonsteroidal synthetic estrogen diethylstilbestrol has also been shown to be carcinogenic in humans [6]. Estrogens are considered to be weak tumor initiators [7–9]. One pathway in the metabolism of estrogens leading to tumor initiation includes formation of 4-catechol estrogens from estradiol (E_2) and/or estrone. These catechol estrogens are further oxidized to the corresponding catechol estrogen quinones. The catechol quinones react with DNA at the N-3 of adenine (Ade) and N-7 of guanine (Gua) to form depurinating adducts [8–11]. These adducts are lost from DNA by destabilization of the glycosyl bond. The apurinic sites generated in the DNA can produce mutations by error-prone repair [11,12].

A similar metabolic activation has been found for the synthetic estrogen hexestrol (HES). The major metabolite of HES is its catechol [3,13], which can be oxidized to the catechol quinone. The HES-3′,4′-quinone (HES-3′,4′-Q) reacts with DNA at the N-3 of Ade and N-7 of Gua to form depurinating adducts analogous to the adducts formed by cat-

Abbreviations: Ade, adenine; dG, 2′-deoxyguanosine; DMF, *N*,*N*-dimethylformamide; E_2, estradiol; E_2-3,4-Q, estradiol-3,4-quinone; Gua, guanine; HES, hexestrol; HES-3′,4′-Q, hexestrol-3′,4′-quinone; MS/MS, tandem mass spectrometry; 4-OHE_2, 4-hydroxyestradiol; 3′-OH-HES, 3′-hydroxyhexestrol; TFA, trifluoroacetic acid

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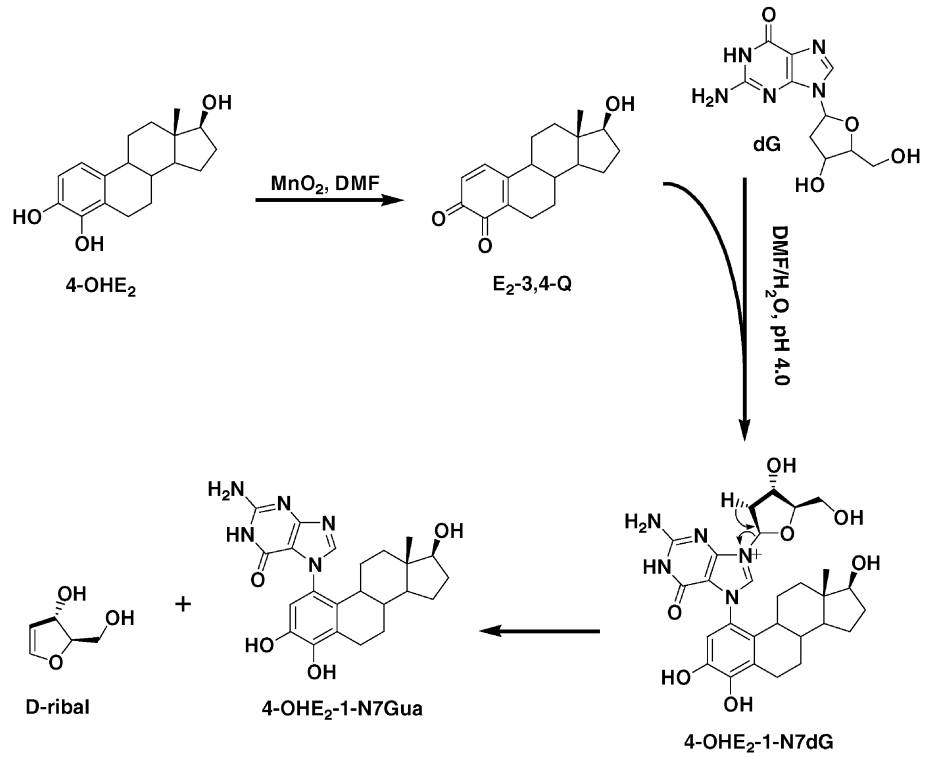

Fig. 1. Reaction scheme for the oxidation of 4-OHE_2 to E_2-3,4-Q and its reaction with dG to form 4-OHE_2-1-N7dG, followed by loss of deoxyribose to yield 4-OHE_2-1-N7Gua.

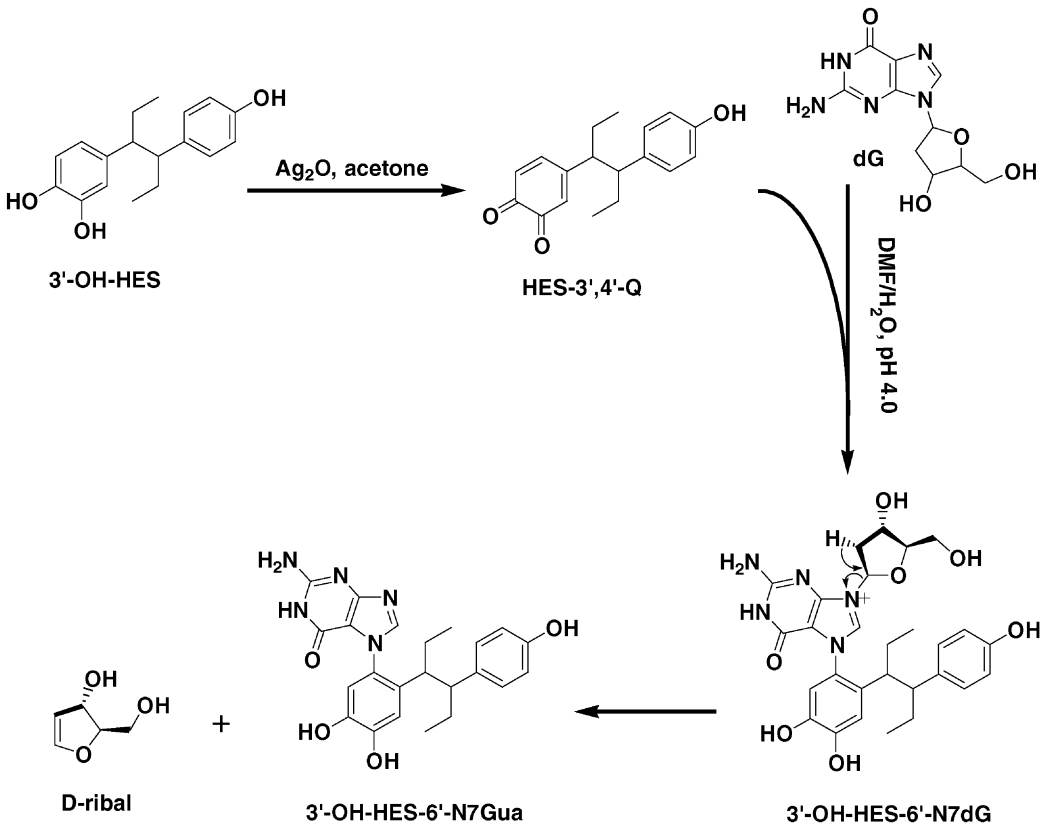

Fig. 2. Reaction scheme for the oxidation of 3′-OH-HES to HES-3′,4′-Q and its reaction with dG to form 3′-OH-HES-6′-N7dG, followed by loss of deoxyribose to yield 3′-OH-HES-6′-N7Gua.

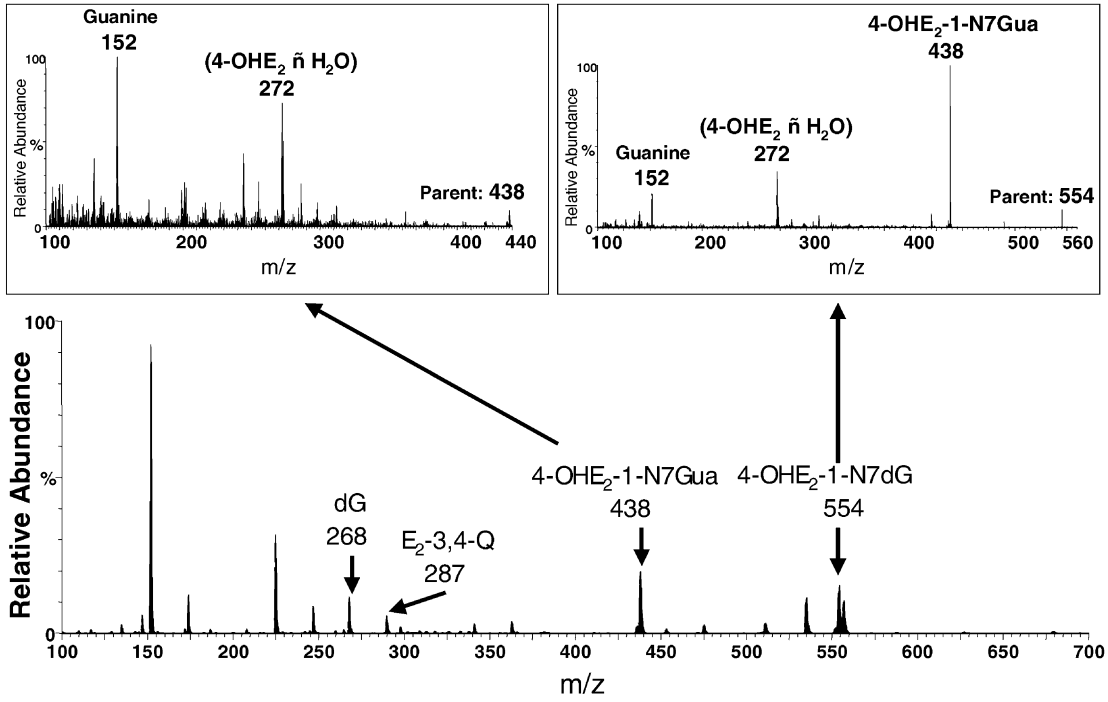

Fig. 3. Mass spectrum of the reaction of E_2-3,4-Q with dG after 3 h at 22 °C. Right inset, MS/MS of 4-OHE_2-1-N7dG; left inset, MS/MS of 4-OHE_2-1-N7Gua.

echol estrogen-3,4-quinones [14,15]. In this article, we report the slow loss of deoxyribose from the N7deoxyguanosine (N7dG) adducts formed by reaction of E_2-3,4-Q or HES-3′,4′-Q with dG (Figs. 1 and 2). This is an additional similarity between the natural estrogen E_2 and the synthetic estrogen HES.

2. Experimental methods

2.1. Chemicals and reagents

Ag_2O and DMSO-d_6 were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2′-Deoxyguanosine (dG) was purchased from USB (Cleveland, OH). 4-Hydroxyestradiol (4-OHE_2) was synthesized as previously described [16] and 3′-hydroxyhexestrol (3′-OH-HES) was synthesized by a new method [17]. Activated MnO_2 was prepared as previously described [18], by treating concentrated aqueous $KMnO_4$ with aqueous $MnSO_4$ solution kept at 90 °C, until a slight excess of $KMnO_4$ was present, as indicated by the pink coloration of the suspension.

3. Instrumentation

3.1. UV

The UV spectra were obtained during HPLC by using a Waters 996 photodiode array detector (Milford, MA). HPLC separations were monitored at 290 nm for 4-OHE_2 and at 277 nm for 3′-OH-HES.

3.2. Mass spectrometry

To verify the presence and identity of the adducts, reaction components were analyzed by direct infusion MS and tandem MS (MS/MS), using a MicroMass QuattroMicro triple quadrupole mass spectrometer (Waters). A 100-μl aliquot of the reaction mixture was diluted 1:1 in triple distilled H_2O, then passed through a 0.22 μm syringe filter prior to infusion into the mass spectrometer. Flow from the syringe was 5 μl/min directed to an interface at 120 °C with nitrogen as both the desolvation and auxiliary gas. Reaction components were ionized in the positive ion mode with the capillary at 3.5 kV and cone at 25 V. Full-scan spectra between 20 and 700 amu were obtained by combining 100 scans. MS/MS conditions were optimized for each individual peak identified in full scan spectra. Argon was used as the collision gas.

3.3. HPLC

Analytical HPLC was conducted on a Waters 2690 Separations Module equipped with a Waters 996 photodiode array detector and a reversed phase Phenomenex Luna-2 C-18 column (250 mm × 4.6 mm, 5 μm; 120 Å, Torrance, CA). Preparative HPLC was conducted on a Waters 600E solvent delivery system equipped with a 996 photodiode

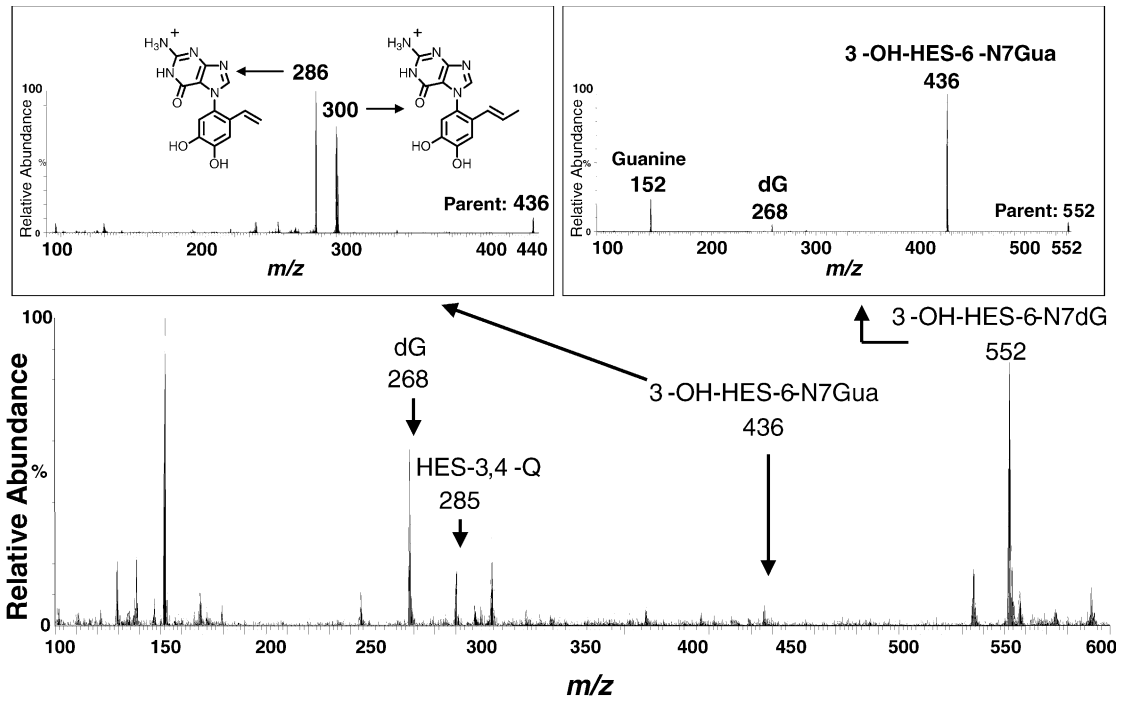

Fig. 4. Mass spectrum of the reaction of HES-3′,4′-Q with dG after 3 h at 22 °C. Right inset, MS/MS of 3′-OH-HES-6′-N7dG; left inset, MS/MS of 3′-OH-HES-6′-N7Gua.

array detector and YMC ODS-AQ 5-μm, 120 Å column (20 mm × 250 mm, Morris Plains, NJ).

3.4. Reaction of E_2-3,4-Q with dG and loss of deoxyribose

To an efficiently stirred suspension of activated MnO_2 (61 mg, 0.70 mmol) in 1 ml of dimethylformamide (DMF) at 0 °C was added a solution of 4-OHE_2 (20 mg, 0.07 mmol) in 1 ml of DMF under argon [19]. After stirring for 20 min, the yellowish-green estradiol-3,4-quinone (E_2-3,4-Q) was filtered quickly through a syringe filter into a solution of dG (99 mg, 0.35 mmol) in DMF/H_2O (4 ml, 1:3). The final solution was DMF/H_2O (1:1). The pH of the solution was adjusted to 4 with acetic acid (200 μl), and the progress of the reaction at room temperature (22 °C) was monitored at different time intervals (0.5, 1, 2, 3, 4, 5, 7, 10, 12, 16, 20, and 24 h). Aliquots at the selected time points were analyzed quickly by using analytical HPLC monitored at 290 nm. The column was eluted starting with 10% CH_3CN in H_2O (0.4% trifluoroacetic acid (TFA) (and then with a linear gradient to 100% CH_3CN in 30 min. The 4-OHE_2-1-N7dG, 4-OHE_2-1-N7Gua, and E_2-3,4-Q eluted at 10.4, 11.1, and 17.3 min, respectively, and were analyzed and quantified at 290 nm by using Millennium™ software. Quantification was accomplished by using standards of E_2-3,4-Q and 4-OHE_2-1-N7Gua; the latter served for both 4-OHE_2-1-N7Gua and 4-OHE_2-1-N7dG, as both compounds showed similar UV spectra. The reactions were run and analyzed in triplicate.

3.5. Reaction of HES-3′,4′-Q with dG and loss of deoxyribose

3′-OH-HES (17 mg, 0.2 mmol) was dissolved in 1.5 ml of acetone and cooled to 0 °C, and 54 mg of Ag_2O was added slowly. The reaction mixture was brought to room temperature (22 °C) and stirred for 30 min. It was mixed with 1 ml of DMF and the acetone was evaporated. The yellowish-green quinone was filtered directly into a solution of dG (83 mg) in DMF/H_2O (5 ml, 2:3) at pH 4.0 with one drop of acetic acid. The final solution was DMF/H_2O (1:1). Formation of the adducts was monitored on the analytical HPLC at 277 nm after 0.5, 1, 2, 3, 4, 5, 7, 9, 11, 17, and 24 h. The column was eluted starting with 10% CH_3CN in H_2O (0.4% TFA) for 5 min at a flow rate of 1 ml/min, followed by a linear gradient to 80% CH_3CN in 30 min. The 3′-OH-HES-6′-N7dG, 3′-OH-HES-6′-N7Gua, and HES-3′,4′-Q were eluted at 17, 18, and 26 min, respectively, and were analyzed and quantified at 277 nm by using Millennium™ software. Quantification was accomplished by using standards of HES-3′,4′-Q and 3′-OH-HES-6′-N7Gua; the latter served for both 3′-OH-HES-6′-N7Gua and 3′-OH-HES-6′-N7dG, as both compounds showed similar UV spectra. The reactions were run and analyzed in triplicate.

4. Results

The Michael addition of dG to E_2-3,4-Q and to HES-3′,4′-Q was conducted at pHs ranging from pH 1 to 7. A pH of 4 was selected for this study because under these conditions the highest yield of N7Gua adducts was obtained (unpublished results).

4.1. 4-OHE_2-1-N7Gua

Reaction of E_2-3,4-Q with dG yielded the labile adduct 4-OHE_2-1-N7dG, which slowly converted into 4-OHE_2-1-N7Gua (Fig. 1). The structure of the latter adduct was previously demonstrated [19]. After 3 h, an aliquot of the reaction mixture was directly infused into the mass spectrometer (Fig. 3) to give the $[M+H]^+$ of 4-OHE_2-1-N7dG, *m/z* 554. The scan of this 3-h mixture also contained a peak at *m/z* 438, corresponding to 4-OHE_2-1-N7Gua, and peaks at *m/z* 287 and 268, corresponding to E_2-3,4-Q and dG, respectively (Fig. 3). The adduct 4-OHE_2-1-N7dG was definitively identified by MS/MS, in which the fragment at *m/z* 438 corresponded to 4-OHE_2-1-N7Gua and the fragment at *m/z* 272 corresponded to the loss of H_2O from 4-OHE_2 (Fig. 3, right inset).

4.2. 3′-OH-HES-6′-N7Gua

Reaction of HES-3′,4′-Q with dG yielded the labile adduct 3′-OH-HES-6′-N7dG, which slowly converted into 3′-OH-HES-6′-N7Gua (Fig. 2). The structure of the latter adduct was previously demonstrated [14]. After 3 h, an aliquot of the reaction mixture was directly infused into the mass spectrometer to give the characteristic molecular ions corresponding to the compounds in the mixture (Fig. 4). The $[M+H]^+$ at *m/z* 552 indicated formation of 3′-OH-HES-6′-N7dG. Fragmentation of this peak by MS/MS yielded the major daughter ion at *m/z* 436, corresponding to 3′-OH-HES-6′-N7Gua, which derived from the parent compound minus deoxyribose (Fig. 4, right inset). Other daughter ions were detected at *m/z* 268 and 152, which corresponded to dG and Gua, respectively. These fragments definitively identified the labile adduct 3′-OH-HES-6′-N7dG. MS/MS of 3′-OH-HES-6′-N7Gua (Fig. 4, left inset) produced the two fragments at *m/z* 300 and 286, which are the fingerprint of this adduct [14].

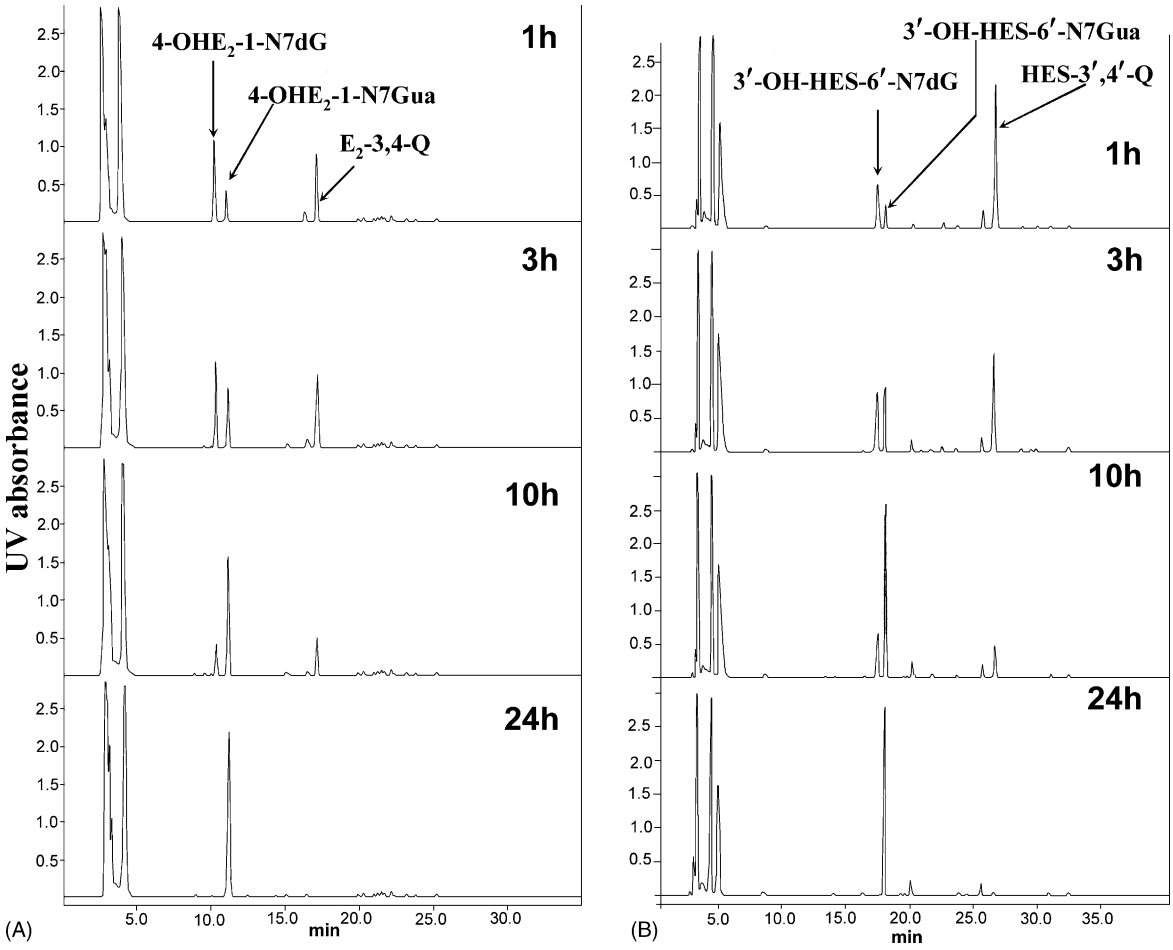

Fig. 5. HPLC profile at various time points of the (A) reaction between E_2-3,4-Q and dG and (B) reaction between HES-3′,4′-Q and dG.

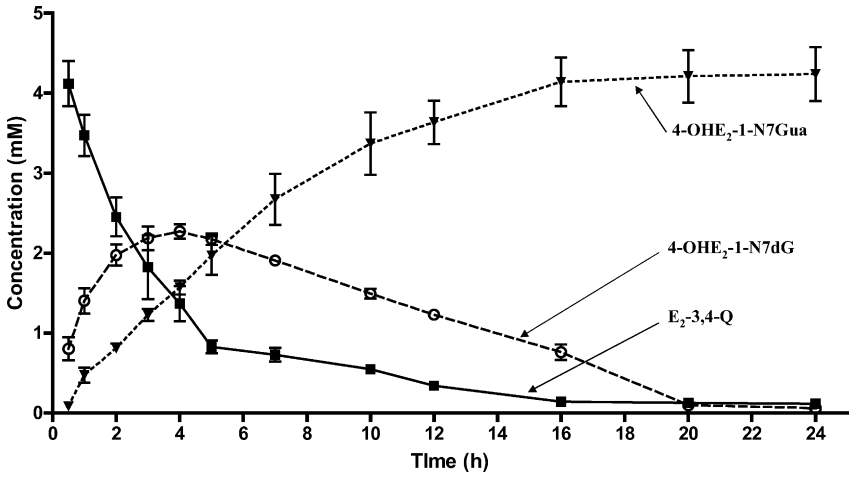

Fig. 6. Formation and disappearance of 4-OHE_2-1-N7dG, formation of 4-OHE_2-1-N7Gua and disappearance of E_2-3,4-Q over time.

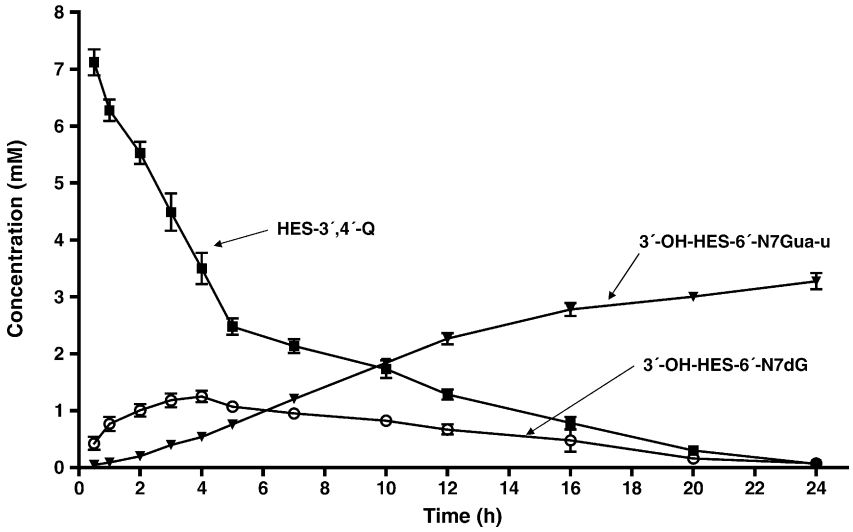

Fig. 7. Formation and disappearance of 3′-OH-HES-6′-N7dG, formation of 3′-OH-HES-6′-N7Gua and disappearance of HES-3′,4′-Q over time.

4.3. *Time course of the reaction*

As indicated in Figs. 1 and 5A, the reaction between E_2-3,4-Q and dG initially yielded 4-OHE_2-1-N7dG via a 1,4-Michael addition of the N-7 of dG to the C-1 of E_2-3,4-Q. In the first 5 h, the concentration of E_2-3,4-Q decreased rapidly, concomittantly with the formation of 4-OHE_2-1-N7dG (Fig. 6). This adduct subsequently released the deoxyribose moiety and was converted to the 4-OHE_2-1-N7Gua adduct (Figs. 5A and 6). The concentration of the 4-OHE_2-1-N7dG increased in the first 4 h, but then decreased because the adduct was converted to 4-OHE_2-1-N7Gua (Fig. 6).

Reaction of HES-3′,4′-Q with dG proceeded in a similar manner, with initial formation of 3′-OH-HES-6′-N7dG via a 1,4-Michael addition of the N-7 of dG to the C-6′ of HES-3′,4′-Q (Figs. 2, 5B and 7). The 3′-OH-HES-6′-N7dG adduct subsequently lost deoxyribose to yield the 3′-OH-HES-6′-N7Gua adduct. The concentration of 3′-OH-HES-6′-N7dG increased for 4 h, but then decreased because the adduct was converted to 3′-OH-HES-6′-N7Gua (Fig. 7).

5. Discussion

The reaction of E_2-3,4-Q or HES-3′,4′,-Q with dG yielded the N7dG adduct, which slowly lost the deoxyribose moiety as a result of destabilization of the glycosyl bond. Formation and decomposition of the N7dG adducts were demonstrated by a combination of HPLC analysis of the reactions at various time points and by MS analysis. The reaction of HES-3′,4′,-Q with dG was approximately 25% less efficient than the reaction of E_2-3,4-Q with dG. The half-life of the N7dG adduct was approximately 6 h for 4-OHE_2-1-N7dG and approximately 8 h for 3′-OH-HES-6′-N7dG. When E_2-3,4-Q or HES-3′,4′,-Q is reacted with DNA, the 4-OHE_2-1-N3Ade or 3′-OH-HES-6′-N3Ade depurinates instantaneously, whereas

the 4-OHE_2-1-N7Gua or 3′-OH-HES-6′-N7Gua is released from the DNA slowly over several hours [15,20].

The biological effects of the slow release of N7Gua adducts from DNA in vivo has been demonstrated with E_2-3,4-Q. When mouse skin was topically treated with E_2-3,4-Q, covalent binding of the quinone to DNA led predominantly to the formation of the depurinating N7Gua and N3Ade adducts in equal amounts [12]. Similar results were obtained when E_2-3,4-Q was administered to rat mammary glands by intramammillary injection [21]. Loss of these depurinating adducts generates apurinic sites that lead to mutations in the H-*ras* oncogene by error-prone repair [12]. Following treatment with the E_2-3,4-Q, mutations were determined in both animal model systems at 6 h, 12 h, 1 d, and 3 d after treatment. Despite the formation of N7Gua and N3Ade adducts in similar amounts, almost all of the mutations were detected at Ade sites, suggesting that the rate of depurination is a critical factor in generating mutations. Therefore, the burden of mutations by fast depurination has been suggested to be a critical factor in the induction of cancer-causing mutations by error-prone misrepair of apurinic sites [12].

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