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Interaction of bisphenol A 3,4-quinone metabolite with glutathione and ribonucleosides/deoxyribonucleosides *in vitro*

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HIGHLIGHTS

- BPAQ was capable of binding directly to the nucleophilic site of ribonucleosides/deoxyribonucleosides *in vitro* via a Michael addition.
- BPAQ produced depurinating adducts that was lost from deoxyribonucleosides, generating apurinic sites in the deoxyribonucleosides.
- ESI–MS/MS is a powerful analytical tool for the detection of suspected ribonucleosides/deoxyribonucleosides and GSH adducts.

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ABSTRACT

Bisphenol A is a monomer used in the manufacture of polycarbonate plastic products, epoxy resin-based food can liners and flame retardants. To determine the genotoxic potential of bisphenol A, the mechanism of the reactions between the reactive electophilic bisphenol A 3,4-quinone (BPAQ) with glutathione and ribonucleosides/deoxyribonucleosides were studied. The obtained results demonstrated that BPAQ reacted with 2′-deoxyguanosine (dG)/guanosine (G), 2′-deoxyadenosine (dA)/adenosine (A), but not with 2′-deoxycytidine (dC)/cytidine (C) and thymidine (T)/uridine (U) in aqueous acetic acid. The reactions were accompanied by loss of deoxyribose, and the rate of depurination by deoxyribonucleoside adducts were faster than that of ribonucleoside adducts. In mixtures of ribonucleosides and deoxyribonucleosides treated with BPAQ, reactions occurred more readily with dG/G than dA/A. The structures of the modified bases were confirmed by electrospray ionization tandem mass spectrometry (ESI–MS/MS). We also found that BPAQ reacted readily with glutathione (GSH) in aqueous acetic acid, and characterized the BPAQ-GSH conjugate by ESI–MS/MS. The *in vitro* data of depurinating DNA/RNA adducts and BPAQ-GSH adducts may provide appropriate reference for the identification of BPAQ adducts in environmental and biological systems.

1. Introduction

Bisphenol A (BPA) is a widely used industrial chemical that can be found as a monomer of polycarbonate plastics and epoxy resins which are used in baby bottles, in the lining of food cans, and in dental sealants. BPA is a weak estrogen-like compound and is therefore regarded as one of the endocrine disrupting chemicals (EDCs) [1]. Although manufacturers and governments claim that BPA is safe and its entry into the environment has no impact on human health [2], the safety/toxicology of BPA is of public concern. Several studies have been performed *in vitro* and *in vivo* concerning its mutagenic, genotoxic, and carcinogenic effects [3]. Peltonen showed that BPA yielded reactive free radicals in occupational photoallergy caused by exposure to the thermal degradation products of epoxies [4]. Comet assays showed that BPA exerted aneuploidy and structural chromosomal aberrations on L5178Y *tk* ± mouse lymphoma [5], ER-positive MCF-7 [6] and CHO-K1 cells [7]. Ames tests showed that BPA induced structural changes (achromatic gaps) in bone marrow cells of mice and the erythrocyte micronucleus assay was also positive in human lymphoblastoid AHH-1 cells *in vitro* [8]. Furthermore, Tsutsui studies showed that BPA induced numerical chromosomal changes in SHE cells [9] and, although much less strong than 17β-estradiol, it caused DNA damage in estrogen receptor (ER)-positive MCF-7 cells. Huff researches showed that formation of DNA adducts in liver cells may be tentatively correlated with hepatocellular toxicity, which was shown by the very high incidence of multinucleated

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hepatocellular giant cells observed in BPA-treated B6C3F1 mice, in the absence of liver tumors [10]. In order to gain additional insight into the genotoxicity of BPA, it is important to understand the mechanism and dynamics of the chemical reaction between the bisphenol A metabolite and DNA.

The first critical step in the BPA-induced cancer initiation may be the formation of covalently bonded carcinogen-DNA adducts [11]. In mammalian cells, BPA can be oxidized to quinones (BPAQ) by peroxidases and cytochrome P450 [12]. It is hypothesized that BPAQ may be the ultimate carcinogenic form of estrogens, because these electrophiles can covalently bind to nucleophilic groups on DNA via a Michael addition. There are two types of adducts formed: (1) stable adducts that remain in DNA unless removed by repair and (2) depurinating adducts that are released from DNA with the formation of apurinic sites. BPAQ react with DNA at the N7 position of guanine (Gua) and the N7 position of adenine (Ade) to form depurinating adducts in most cases [13]. The apurinic sites generated in the DNA can produce mutations by error-prone repair [10,11]. Evidence showed that depurinating BPAQ-DNA adducts play a major role in cultured human prostatic cells [14–18]. In the case of BPAQ-DNA adducts, different results were reported. Edmonds et al. working on DNA and deoxyguanosine (dG) reported that no stable adduct, but only the depurinated guanine product was obtained [19]. Other studies, however, have shown the presence of dG or DNA adducts [2,20,21].

While the formation of DNA adducts has been studied, few study have been conducted with RNA. Since RNA has a similar structure to DNA, RNA was expected to show similar reactivity to electrophiles. Emerging evidences have resulted in the discovery that cellular RNA could be modified upon exposure to external stimuli such as toxicants [22–24]. Compared with DNA, RNA is not usually as densely folded, so it may be more susceptible to reaction with drugs. These evidences suggest that RNA is a target of electrophiles in a manner similar to DNA.

Glutathione (GSH) is the most important endogenous scavenger. In its reduced form, GSH can react with a variety of electrophiles and radical species *in vivo* [25]. Therefore, conjugation of BPAQ with GSH would constitute a detoxification pathway that competes with reactions of BPAQ with DNA. A computational study of the reactivity of BPAQ with deoxyadenosine and GSH suggested that BPAQ preferably reacts with GSH and only with DNA when the level of GSH in the cell is low [26]. Detailed knowledge of the reaction mechanism is the key to understanding the possible genotoxicity of BPA on the molecular level. Therefore, it is important to study the bonding of GSH with BPAQ for better understanding the BPA toxicological mechanism.

To understand BPA at the genome level and to shed light on the mechanisms of reaction with ribonucleosides/deoxyribonucleosides and GSH *in vivo*, effective analytical method is needed to perform both qualitative and quantitative measurements on the formed adducts. Compared with ^{32}P-post labeling, mass spectrometry (MS) is a powerful tool for analyzing structure of ribonucleosides/deoxyribonucleosides adducts. In particular, liquid chromatography/mass spectrometry (LC/MS) is capable of detecting and distinguishing ribonucleosides/deoxyribonucleosides adducts due to the ability to separate compounds/mixtures by LC and to subsequently determine their molecular weights and fragmentation by using tandem MS. High resolution mass spectrometry, for example, quadrupole time-of-flight MS (Q-TOF-MS), allows confirmation of the detected ions with accurate mass measurement. Ultrahigh performance liquid chromatography/mass spectrometry (UPLC/MS) is particularly suitable for quantitative analysis with low detection limits. The primary objective of our study is to investigate the formation of ribonucleosides/deoxyribonucleosides and GSH adducts by reacting with BPAQ.

2. Material and methods

2.1. Chemicals and reagents

2′-Deoxyguanosine (dG), 2′-deoxyadenosine (dA), guanosine (G), adenosine (A), 2′-deoxycytidine (dC), thymidine (T), cytidine (C), uridine (U), L-GSH and potassium nitrosodisulfonate (Fremy's salt) were obtained from Sigma (St. Louis, MO, USA). Bisphenol A (BPA) was obtained from Tokyo Chemical Industrial Co., Ltd. (Tokyo, Japan). $CDCl_3$ was purchased from Acros Organics (Geel, Belgium). Precoated Thin Layer Chromatographic (TLC) plates (DC-Fertigplatten SIL G-25 UV254) were purchased from Macherey–Nagel (Düren, Germany). Silica gel 60 (0.040–0.063 mm for column chromatography) was obtained from Merck, Darmstadt, Germany. Deionized water was purified by employing a Milli-Q reagent water system (Millipore, Billerica, MA, USA).

2.2. Synthesis of BPA-3,4-quinone

BPA 3,4-quinone (BPAQ) was synthesized according to the method of Atkinson and Roy with minor modifications [20]. BPA (100 mg, 0.44 mmol in 33 mL acetone), was oxidized with potassium nitrosodisulfonate (1.8 g, 6.7 mmol, in 180 mL of 10% acetic acid). Vigorous shaking was applied after each addition (15 min per time) at room temperature. The mixture was extracted with 2 vols of chloroform, and washed 3 times with 5% HCl, water and saturated NaCl solution. The extract was evaporated to dryness under a stream of nitrogen and finally suspended into a small volume of chloroform. The crude BPAQ was purified by using a silica gel column (5 g silica gel) and isocratic elution (hexane:ethyl acetate, 3:1). Each fraction (50 mL) was monitored on a TLC plate, using the same solvent. The fractions containing pure BPAQ were combined and concentrated under vacuum to afford a brown amorphous powder (yield of about 20%). The identity of the BPAQ was established by mass spectrum and NMR spectrum. Mass spectrum of the preparation showed evidence for the mass of BPAQ, $[M\text{-}H]^-$ ion at m/z 241 (Fig. 1). Comparison of the detected protonated molecular ions of adducts from ESI-QTOFMS analysis with the corresponding theoretical values is presented in Table S1, Supporting information. ^{1}H NMR ($CDCl_3$): δ1.54(6H, s, $-CH_3 \times 2$), 6.22 (1H, dd, J = 10.3, 0.7 Hz, H6), 6.50 (1H, dd, J = 2.4, 0.7 Hz, H3), 6.64 (1H, dd, J = 10.3, 2.4 Hz, H5), 6.84 (2H, d, J = 8.8 Hz, H3′, H5′), 7.14 (2H, d, J = 8.8 Hz, H2′, H6′), ^{13}C NMR ($CDCl_3$): δ 27.26, 43.03, 115.89, 123.76, 127.79, 128.99, 136.02, 141.60, 154.83, 161.46, 180.397, 180.404.

2.3. Reaction of BPAQ with ribonucleosides/deoxyribonucleosides

BPAQ (60 μg, 0.25 μmol) was dissolved in 62.5 μL of acetone and then reacted in separate experiments with 250 μL of dG (355 μg, 1.25 μmol), G (357 μg, 1.25 μmol), dA (340 μg, 1.25 μmol), A (335 μg, 1.25 μmol), dC (285 μg, 1.25 μmol), C (305 μg, 1.25 μmol), T (304 μg, 1.25 μmol), U (306 μg, 1.25 μmol) solution in AcOH-H_2O (1:1). The mixtures were kept at 37 °C for 24 h. An aliquot of 10 μL from each incubation solution was diluted with the spray solvent (50/50 V/V acetonitrile/H_2O) to 200 μL for ESI–MS.

ESI–MS/MS data were acquired on a Q-TOF mass spectrometer (API QStar Pulsari, Applied Biosystems, Foster City, CA, USA) equipped with a turbo ion spray source in positive ion mode. Experiments were conducted with an ion spray voltage of 5200 V, declustering potential of 52 V, focusing potential of 220 V and declustering potential II of 10 V. The scanning mass range was between m/z 90 and 600. The ion source port and the curtain gas were set at 20 and 25 lb, respectively. The reaction samples were

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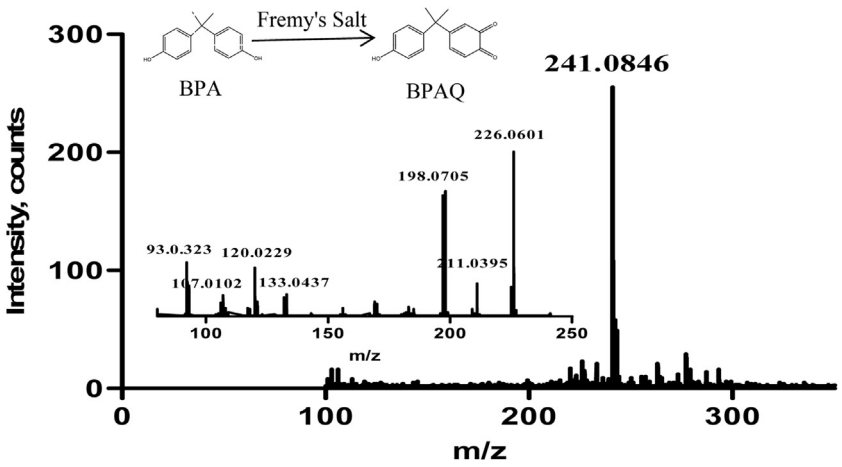

Fig. 1. MS/MS spectra of the $[M-H]^-$ ion of BPAQ.

injected directly into the ESI source by using an infusion pump with flow rate of 10 mL min^{-1}. Data acquisition and processing were performed by using Analyst QS software.

2.4. Rate of depurination of 3-OH-BPA-N7dG and 3-OH-BPA-N7G

The progress of the reaction at 37 °C was monitored at different time intervals (0.5 h, 5 h, 24 h, 72 h, 96 h, 120 h and 144 h). An aliquot of 10 μL from each incubation solution was diluted in 200 μL of acetonitrile/H_2O (50/50, V/V) for UPLC/MS analysis.

Quantitative analysis of DNA adducts was performed with multiple reaction monitoring (MRM) by using triple-quadrupole mass spectrometry equipped with an ESI source in the positive ion mode (Waters ACQUITY TQ Detector, Waters Corporation, Milford, MA, USA). The 3-OH-BPA-N7dG and 3-OH-BPA-N7 G adducts were separated by a UPLC (Waters ACQUITY UPLC system, Waters Corporation, Milford, MA, USA) with a Waters Acquity reversed-phase bridged ethylene hybrid phenyl column (2.1 mm × 150 mm, 1.7 μm). Mobile phase was consisted of two components: A (0.2% aqueous acetic acid) and B (ACN), and held at 95% A for 2 min before beginning the gradient program. A gradient program was used as 95% A and 5% B followed by a linear gradient to 65% A and 35% B over the course of 6 min and then a linear gradient to 5% A and 95% B for another 2 min. The column was then allowed to re-equilibrate back to the starting mobile phase of 95% A for 2 min before the next injection. An injection volume of 10 μL was selected with a flow rate of 0.3 mL min^{-1}. Optimized MS parameters were described as follows: capillary voltage was 3000 V; dwell time was 0.1 s; extractor voltage was 3 V; temperatures of ESI source and desolvation gas were 118 and 300 °C, respectively; cone gas and desolvation gas flows were 40 and 400 L h^{-1}, respectively. MRM mode was employed with the following *m/z* transitions for the target analytes: 3-OH-BPA-N7G, 526 > 394; 3-OH-BPA-N7Gua, 394 > 260; BPAQ, 243 > 91. Instrument operation and data acquisition were processed by using the Waters MassLynx V4.1 SCN562 software package.

2.5. Reaction of BPA 3,4-Q with reduced GSH

The reaction of BPAQ with reduced GSH was conducted as follows. A 20 μL of GSH solution (0.3 μmol in distilled water) was reacted with 20 μL of BPAQ solution (0.3 μmol in acetone) for 24 h at 37 °C. The crude product was diluted in 400 μL of acetonitrile/H_2O (50/50, V/V) and analyzed by ESI–MS via direct infusion.

The BPAQ-GSH adducts were separated by UPLC (Waters ACQUITY UPLC system, Waters Corporation, Milford, MA, USA) with a Waters Acquity reversed-phase ethylene bridged hybrid phenyl column (2.1 mm × 150 mm, 1.7 μm). The LC and MS conditions are the same as those described in section 2.4. All data were acquired in positive ion mode with the scan mass range of *m/z* 100–700.

3. Results and discussion

3.1. Synthesis and characterization of BPAQ

BPAQ was synthesized from the oxidation of BPA. The product was analyzed by using ESI-Q-TOFMS and NMR. The ^1H NMR spectrum showed a signal pattern of a 1,2,4-trisubstituted benzene in addition to an A2 × 2 pattern of a four-substituted phenol, which also pertains to the ^1H NMR of BPA. Besides these typical ^1H NMR signal patterns, two carbonyl signals in the ^{13}C NMR confirmed that this oxidative product did not contain a catechol (3,4-hydroquinone) but a 3,4-quinone moiety.

3.2. Reaction of BPAQ with ribonucleosides/deoxyribonucleosides

To investigate the possible mechanism for the formation of BPAQ-DNA and BPAQ-RNA adducts, BPAQ was allowed to react with four types of deoxynucleoside (dC, dA, dG, T) and four types of nucleoside (C, A, G, U), respectively.

The obtained results indicated that DNA/RNA adducts with dA/A and dG/G could be formed via Michael addition. The adducts were detected from ESI-Q-TOFMS analysis with the information of molecular ions associated with accurate mass measurement and subsequently confirmed by MS/MS analysis. Fig. 2 and Fig. 3 summarize the reactions of BPAQ and ribonucleosides/deoxyribonucleosides. Due to the steric hindrance of phenoxyl group, the Michael addition could not take place between BPAQ and dC/C or T/U, and thus only gradual decomposition of the quinone was observed. Comparison of the detected protonated adducts molecules from ESI-Q-TOFMS analysis with the corresponding theoretical values is presented in Table 1.

Reaction of BPAQ with dG yielded the labile adduct 3-OH-BPA-N7dG, which quickly converted into 3-OH-BPA-N7Gua. After 24 h, an aliquot of the reaction mixture was directly infused into the Q-TOF mass spectrometer (Fig. 4A) to give the $[M+H]^+$ ion of 3-OH-BPA-N7Gua at *m/z* 394. The result was consistent with UPLC/MS/MS analysis, which revealed only the peak of 3-OH-BPA-N7Gua at 5.59 min, indicating that the glycosidic bond underwent rapid hydrolysis under the incubation conditions. The formula of 3-OH-BPA-N7Gua was assigned on the basis of the accurate mass measurements (Table 1). The ion structures in the figure were spec-

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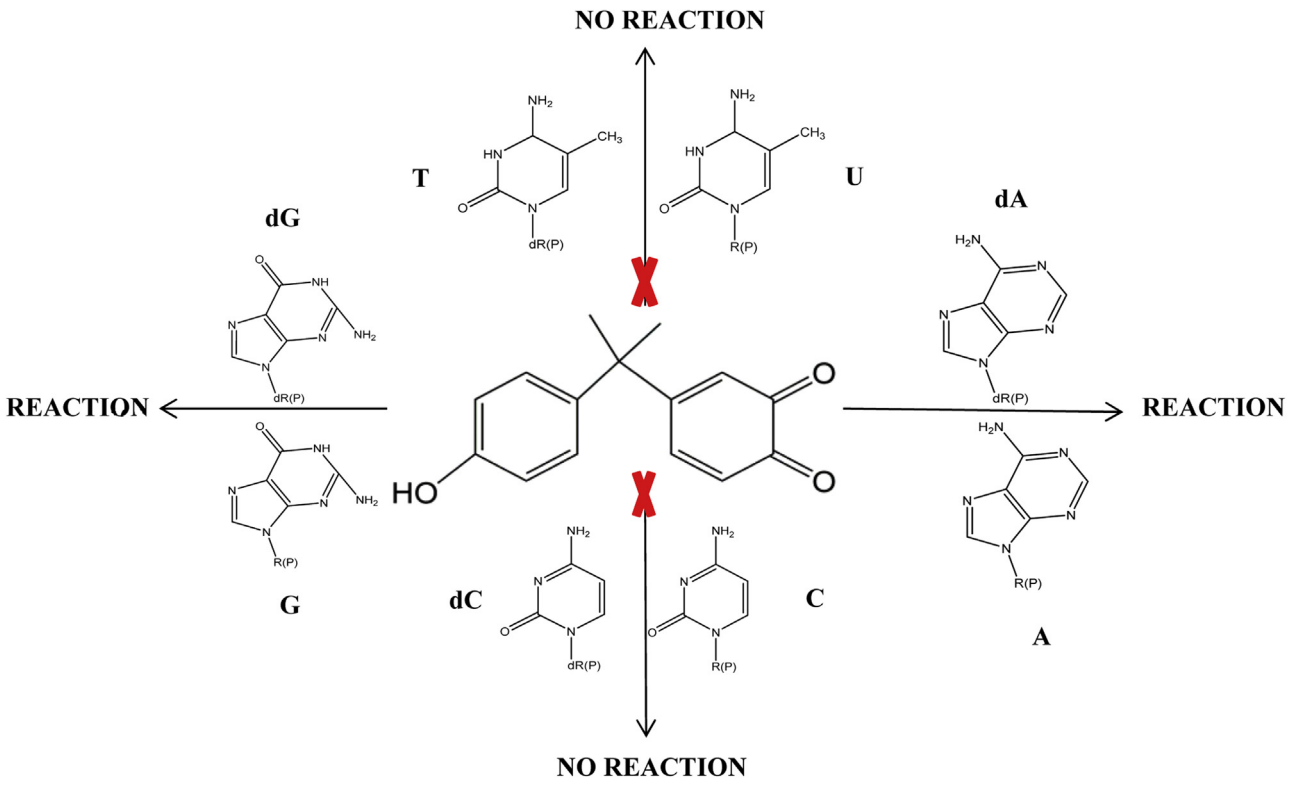

Fig. 2. Reaction of BPAQ with ribonucleosides/deoxyribonucleosides.

Table 1
Accurate mass measurement on protonated molecular ions of the adducts of BPAQ and ribonucleosides/deoxyribonucleosides from ESI-Q-TOFMS analysis.

Name	Formula	Experimental mass	Calculated mass	Error (ppm)
3-OH-BPA-N7G	$C_{25}H_{27}N_5O_8$	526.1958	526.1938	3.8
3-OH-BPA-N7Gua	$C_{20}H_{19}N_5O_4$	394.1548	394.1515	8.37
3-OH-BPA-N7dA	$C_{25}N_5O_6H_{27}$	494.2012	494.2039	5.46
3-OH-BPA-N7A	$C_{25}H_{27}N_5O_7$	510.1947	510.1988	8.04
3-OH-BPA-N7Ade	$C_{20}H_{19}N_5O_3$	378.1543	378.1566	6.08

ulative but with reasonable assignments. For example, a fragment of *m*/*z* 152.0559 (calcd 152.0573 for $C_5H_6N_5O$, protonated guanine) confirmed the presence of a guanine moiety and another of *m*/*z* 243.1045 (calcd 243.1021 for molecular formula of $C_{15}H_{15}O_3$) confirmed the presence of the quinone moiety. As depicted in Fig. 4B, other fragments of *m*/*z* 300.1078 (calcd 300.1097 for $C_{14}H_{14}N_5O_3$), 260.0802 (calcd 260.0784 for $C_{11}H_{10}N_5O_3$), and 135.0834 (calcd 135.0810 for $C_9H_{11}O$) were consistent with losses from either the guanine or the *o*-quinone moiety of the product. Considering steric effects, the complementary product ion pair of *m*/*z* 260 and 135 suggested a linkage between the guanine and the 3,4-di-OH-aromatic ring, mostly likely at the C-5 position, although we could not rule out possibility of other positions (C-2 and C-6). The product ion at *m*/*z* 300, generated from loss of the hydroxylbenzene unit of BPA, further supported the attachment of the 3,4-di-OH-aromatic ring to the guanine moiety. According to Kolšek et al. [26], the activation free energies of Michael addition are lower for N7 dG nucleophilic attack than N3. Both solvation models confirmed that the reaction at the N7 site was more favored. These results provided strong evidence that BPAQ reacted preferentially with the N7 rather than the N3 site. Also, the mass spectrometry study of Qiu et al. [2] showed that the dG N7-position was most probably involved in the reaction with BPAQ.

Reaction of BPAQ with G yielded the labile adduct 3-OH-BPA-N7G, which slowly converted into 3-OH-BPA-N7Gua. After 24 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. S1, Supporting information). The $[M+H]^+$ ion at *m*/*z* 526 indicated the formation of 3-OH-BPA-N7G. Fragmentation of this peak by MS/MS yielded the major daughter ion at *m*/*z* 394, corresponding to 3-OH-BPA-N7Gua, which derived from the parent compound minus deoxyribose (Fig. S1C, Supporting information). MS/MS of 3-OH-BPA-N7Gua (Fig. S1B, Supporting information) produced the fragments at *m*/*z* 135, 243, 260 and 300, which are the fingerprint of this adduct [2]. Analysis of the resulting incubation mixture by UPLC/MS/MS appeared as two peaks eluting at 5.05/5.56 min, respectively, which presumably represent isomeric intermediates and isomeric depurinating adducts (Fig. S4, Supporting information).

We incubated the freshly synthesized BPAQ in parallel with dA and A in 37 °C for 48 h, as previously described for dG and G. The reactions occurred more slowly than those with dG and G, as was seen by the slow losses of color of the starting material BPAQ and the intensity of MS analysis. It is in accordance with the computational study of Kolšek et al. [26], which shows that, comparison with the activation energy for the dA reaction indicates that most of the DNA damage by BPAQ will occur at the guanine cite. Compare to the results observed for the incubation with dG, both intermediates and depurinating adducts were found in deoxyadenosine adducts (Fig. S2, Supporting information). A protonated molecule of *m*/*z* 494 is an unstable intermediate of 3-OH-BPA-N7-dA, which ultimately depurinates to give the 3-OH-BPA-N7-adenine (*m*/*z* 378 [M+H-deoxyribose]$^+$). Unexpectedly, MS/MS on the Q-TOF gave ions of *m*/*z* 136.0613 (calcd 136.0623 for $C_5H_6N_5$), *m*/*z* 252.1054 (calcd 252.1018 for $C_{10}H_{14}N_5O_3$) as the only significant fragments for 3-OHBPA-N7-adenine and 3-OH-BPA-N7-dA, respectively, which confirmed the presence of the adenine moiety. The N7 position of dA has also been considered as the most common binding site

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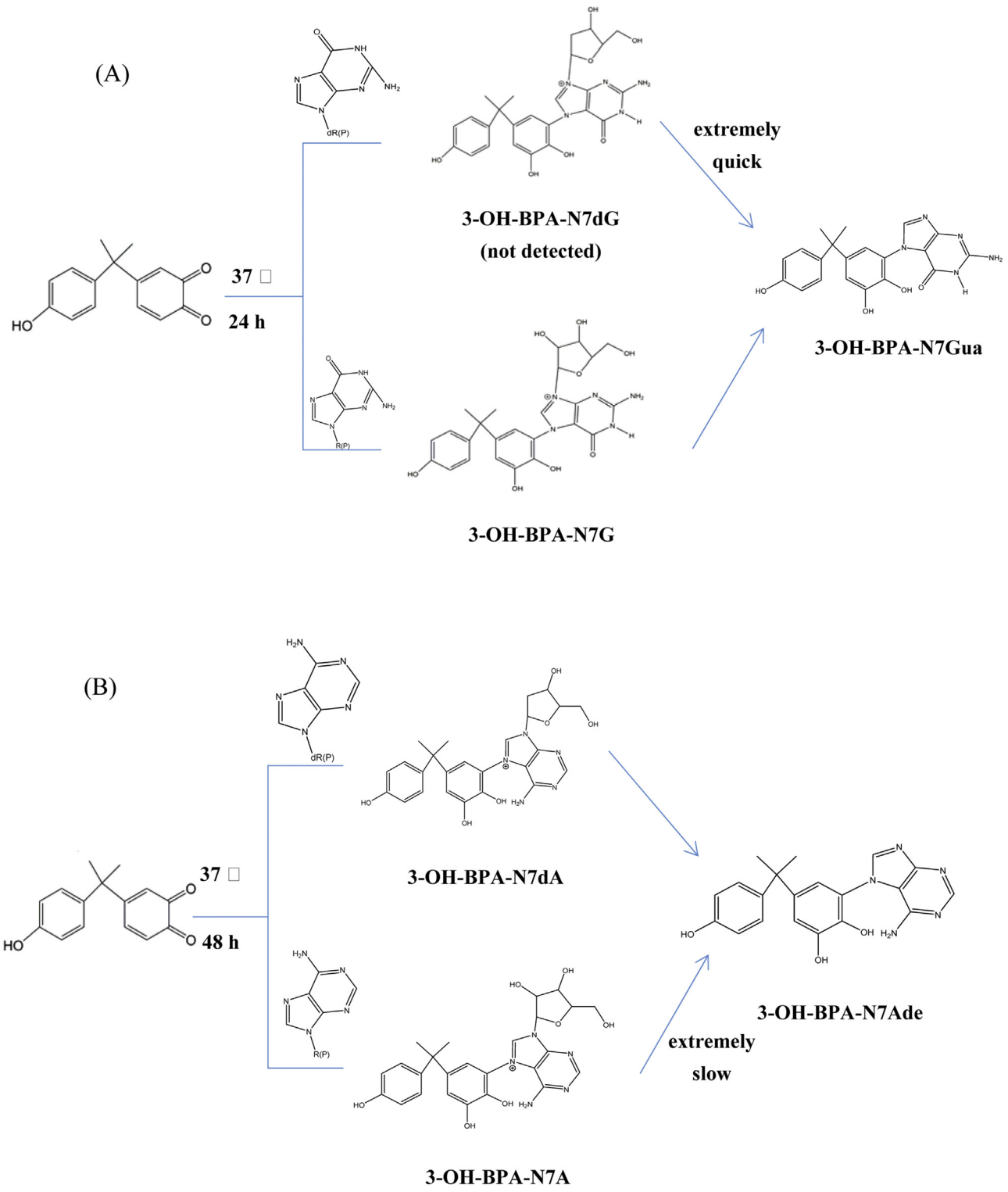

Fig. 3. Reaction of BPAQ with G/dG and rate of depurination (A); Reaction of BPAQ with A/dA and rate of depurination (B).

because of its relatively strong nucleophilic character. The calculated activation barrier for Michael addition is lower for N7 dA nucleophilic attack than N3, which suggested that most of the BPAQ would react with the N7-reactive site of dA to produce 3-OH-BPA-N7-dA [26]. As for the adenosine adducts (Fig. S3, Supporting information), the only detected adduct was 3-OH-BPA-N7A after 48 h incubation, indicating that the depurination of A is much slower than those of dA, dG and G.

3.3. Time course of the reaction

The identified adducts were also analyzed by using UPLC/MS/MS in MRM mode with the selection of ion transition. The obtained results agreed with those from Q-TOFMS and MS/MS analysis, which again confirmed the detection of the produced adducts of BPAQ and deoxynucleosides. The reaction adducts were well sep-

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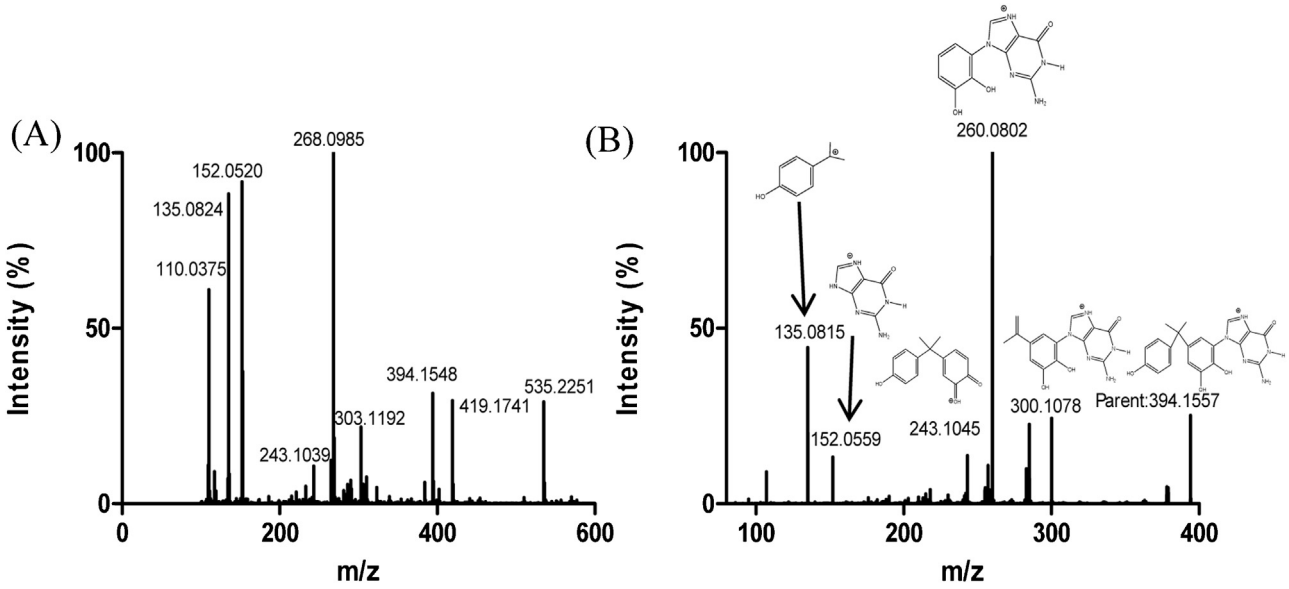

Fig. 4. Mass spectrum of the reaction of BPAQ with dG after 1 d at 37 °C (A), MS/MS of 3-OH-BPA-N7Gua (B).

arated and shown in individual chromatogram with the selected MRM ion channels.

As indicated in Fig. S4, Supporting information, the reaction between BPAQ and G initially yielded 3-OH-BPA-N7G via a 1,4-Michael addition of the N-7 of G to the C-5 of BPAQ, the 3-OH-BPA-N7G adduct subsequently lost deoxyribose to yield the 3-OH-BPA-N7Gua adduct. The concentration of 3-OH-BPA-N7G increased for 72 h, but then decreased because the adduct was converted to 3-OH-BPA-N7Gua (Fig. 5).

Reaction of BPAQ with dG proceeded in a similar manner, but 3-OH-BPA-N7dG could not be detected even in 30 min (data not show), indicating that its rate of depurination was too fast. In the first 24 h, the concentration of BPAQ decreased rapidly, concomitantly with the formation of 3-OH-BPA-N7Gua (Fig. S4, Supporting information).

3.4. Characterization of the BPAQ-GSH adduct

BPA reacts readily with reduced GSH, as seen by the rapid loss of the brown color of BPAQ. The resulting mixture, as analyzed by using UPLC–MS in a manner similar to that for the dG adducts, produced one major GSH conjugate (Fig. S5, Supporting information). The structure of this conjugate was determined by ESI-Q-TOFMS by detecting the protonated molecule at m/z 550.1870 (calcd 550.1859 for $C_{25}H_{32}N_3O_9S$), corresponding to the 3-OH-BPA-mono-GSH conjugate.

Fig. 6 is the MS^2 product-ion spectra of m/z 550. The interpretation of Q-TOFMS/MS spectra for the fragmentation of adducts could help with better understanding on the adduct formation mechanism. The most abundant product ion of m/z 275.0770, with an elemental composition of $C_{15}H_{15}O_3S$, corresponds to $[3\text{-OHBPA-SH}]^+$. It contains the sulfur of the GSH moiety and reveals some structural information about the BPA moiety. m/z 421 and m/z 475 are common diagnostic ions to GSH adducts [27–29]. m/z 135 is likely to arise by the cleavage of the bond linking the two aromatic rings accompanied by a loss of $HS\text{-}C_6H_6\text{-}(OH)_2$. Another confirmation for the depurinating adducts comes from the Q-TOF experiment in which we showed that the elemental composition of the m/z 135 ion is $C_9H_{11}O$.

4. Discussion

BPA is a weak estrogen. As the routes for human exposure to this compound exist, it presents a possible health hazard. Thus, following the pattern of other synthetic estrogens (e.g hexestrol and diethylstilbestrol), BPA also has the potential to cause estrogenic endocrine disruption [30]. However, the mechanism of how BPA elicits these adverse effects is not clear. The catechol pathway in the metabolism of BPA can lead to ultimate carcinogenic species, namely, BPA-*o*-quinone. Motivated by the need for understanding the pathways for chemical and biological conversion of BPA and the causes for its toxicity, we examined the reaction of BPAQ with ribonucleosides/deoxyribonucleosides and GSH with the purpose of providing insight into the possible modes of binding of these intermediates to DNA/RNA.

The present investigation of the reactivity of BPAQ with ribonucleosides/deoxyribonucleosides used UPLC/MS/MS to provide direct evidence that BPA, after metabolic activation to a reactive quinone, reacted with ribonucleosides and deoxyribonucleosides to form adducts. When BPA-Q reacted with dG and dA, the depurinating adduct 3-OH-BPA-N7-Gua and 3-OH-BPA-N7-Ade aroused quickly, presumably via an intermediate 3-OH-BPAN7-dG and 3-OH-BPAN7-dA. This indicates that modified DNA can undergo glycosidic hydrolysis (depurination) to release the modified nucleobase. BPAQ binds inefficiently to RNA, and the resulting adducts depurinate at a very slow rate from RNA. The extremely slow rate of depurination in RNA is due to the higher-energy transition state in the formation of the oxocarbenium ion. This is a consequence of the partial positive charge on the C2′ atom due to the presence of the strongly polarized C2′—O bond of the C2′ hydroxyl group in ribose. Demonstration of the mechanism of depurination might provide us information to explain the results obtained in a variety of studies of BPA and estrogen adducts formed with DNA and RNA [31–34], including the depurinating estrogen-DNA adducts observed in humans [35,36]. Understanding why the depurinating estrogen-DNA adducts found in humans come from DNA, and not RNA, underscores the critical role of these adducts in cancer initiation.

The detoxification reactions in cells compete with reactions between BPAQ and DNA. One of these reactions, with a similar mechanism, is the conjugation of BPAQ with GSH. *In vivo*, GSH reactions are usually promoted by the enzyme glutathione

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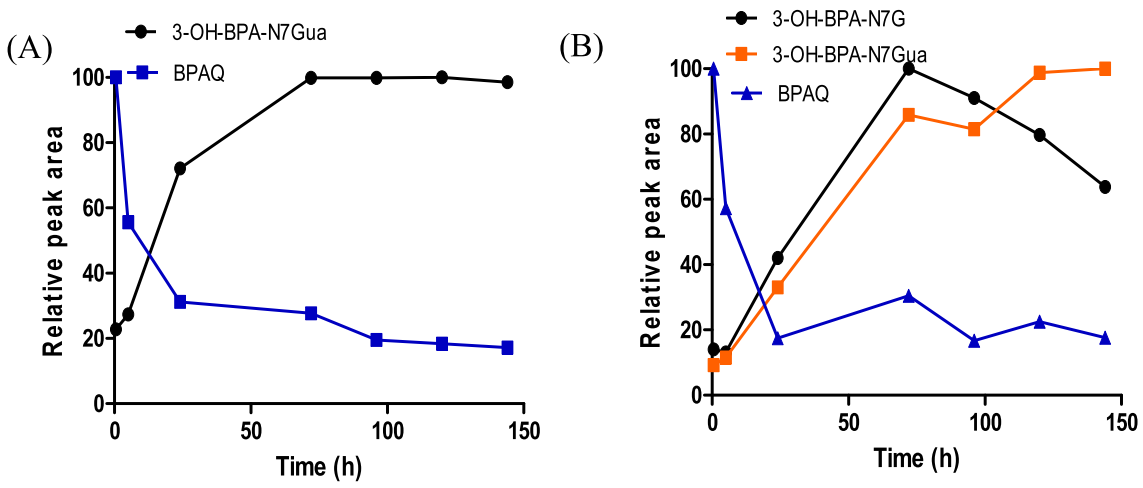

Fig. 5. Formation and disappearance of 3-OH-BPA-N7Gua and BPAQ (A), formation and disappearance of 3-OH-BPA-N7G, 3-OH-BPA-N7Gua and BPAQ (B) at various time points.

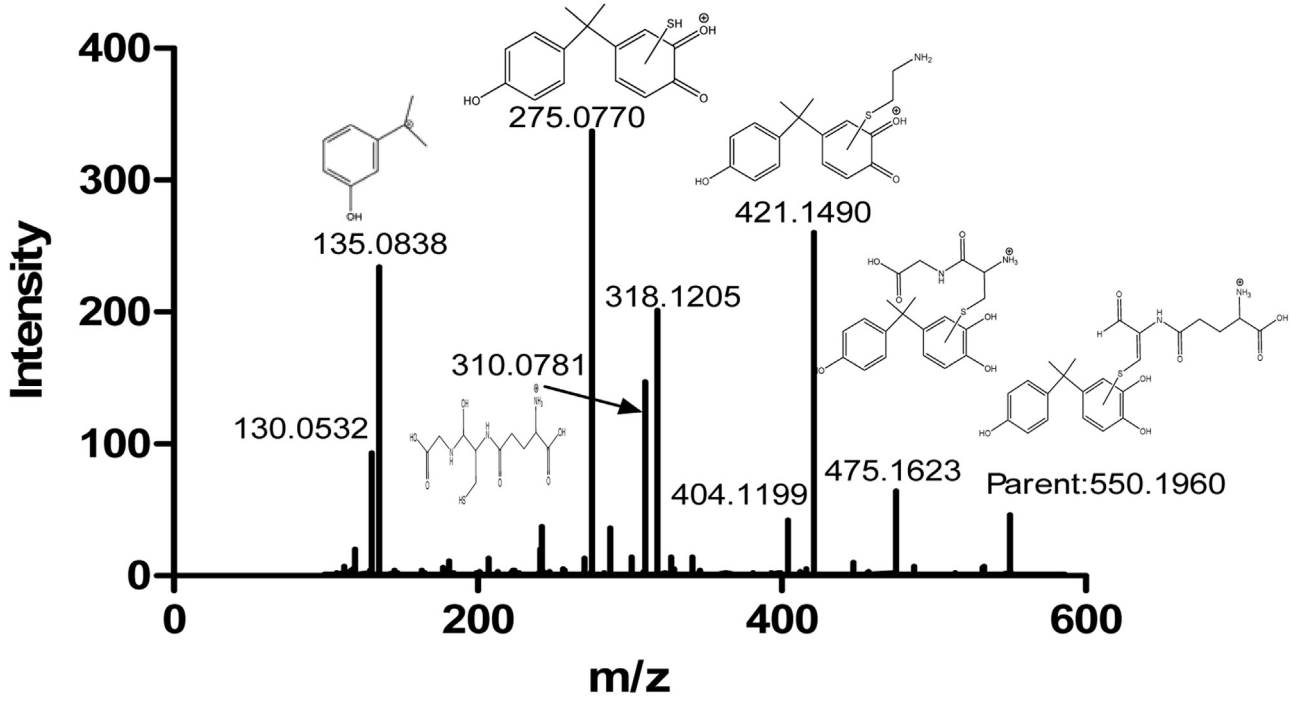

Fig. 6. MS/MS spectrum of the $[M+H]^+$ ion at m/z 550 from 3-OH-BPA-GSH and proposed fragment ion structures.

S-transferase, but in some cases, GSH can react spontaneously without the enzyme, leading to their ultimate removal [37]. The BPAQ-GSH adducts with greater polarity and solubility in water can easily be excreted [2]. Therefore, BPAQ-GSH adducts can be used as potential biomarkers to assess the exposure levels of BPA and the related toxicity. The ability to detect both classes of products is important for developing an understanding of carcinogenesis and for estimating the risk associated with the exposure to BPA. Furthermore, adding reactive oxygen species scavengers might be another efficient way to inhibit BPA oxidizing to BPAQ, which attenuated BPAQ induced toxicity *in vivo* [37–39].

5. Conclusion

The results obtained from the current study demonstrated that BPAQ was capable of binding directly to the nucleophilic site of ribonucleosides/deoxyribonucleosides *in vitro* via a Michael addition. Moreover, BPAQ produced depurinating adducts that was lost from deoxyribonucleosides, generating apurinic sites in the deoxyribonucleosides, which might give potential insight that the depurinating adducts mainly aroused from DNA but not RNA. Our data also suggests that ESI–MS/MS is a powerful analytical tool for the detection of suspected ribonucleosides/deoxyribonucleosides and GSH adducts. The detection of adducts from BPAQ and ribonucleosides/deoxyribonucleosides and GSH might provide valuable information for future studies on BPAQ-DNA/BPAQ-RNA and BPAQ-GSH adducts for a better understanding of biological significance related to the mechanism of chemical toxicology of BPA.

Novelty statement

It is the first time to report how BPAQ reacts with ribonucleosides and compare the situation with that of deoxyribonucleosides.The results obtained from the current study demonstrated

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that BPAQ was capable of binding directly to the nucleophilic site of ribonucleosides/deoxyribonucleosides *in vitro* via a Michael addition. Moreover, BPAQ produced depurinating adducts that was lost from deoxyribonucleosides, generating apurinic sites in the deoxyribonucleosides, which might give potential insight that the depurinating adducts mainly aroused from DNA but not RNA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2016.03.015.

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