Mutagenic activities and physicochemical properties of selected nitrobenzanthrones

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Mutagenic activity of nine nitro derivatives of benzanthrone, namely 1-nitro-, 2-nitro-, 3-nitro-, 9-nitro-, 11-nitro-, 1,9-dinitro-, 3,9-dinitro-, 3,11-dinitro- and 3,9,11-trinitrobenzanthrone were tested with Salmonella strains TA98, TA100, YG1021 and YG1024 in both the presence and absence of an S9 mix. Each compound exhibited mutagenic activity with all the strains. Among these nine isomers, 3-nitrobenzanthrone exhibited the most mutagenic activity with all the strains without the S9 mix. The mutagenic activities of the dinitro and trinitro derivatives of benzanthrone were lower than that of the 3-nitro derivative; this is evident from the mutagenic activity of nitrated polyaromatic hydrocarbons (PAH), which is generally enhanced with an increase in nitration. The physicochemical properties of nitrated benzanthrone (reduction potential, hydrophobicity and orientation of nitro groups to the aromatic ring) demonstrated that mononitrated benzanthrone exhibits a lower reduction potential than mononitroPAHs such as 1-nitropyrene and 3-nitrofluoranthene, but was almost equivalent to that of dinitroPAH. Moreover, the mutagenic activity of mononitrobenzantrones clearly depend on the reduction potential of each compound; however, this tendency was not observed in polynitrobenzanthrones, probably because the reduction of the nitro groups to amino groups of polynitrated benzanthrone might be predominant without a sufficient formation of corresponding hydroxamines. These results suggest that aromatic compounds that contain keto groups, when nitrated, may act as potentially powerful direct-acting mutagens.

Introduction

Aromatic nitro compounds are universally considered to be contaminants in an atmospheric environment and are notably present in automobile exhaust particulates, airborne particles and soot formed after wood combustion (1–4). They are also formed from their parent compounds by atmospheric transformations with nitrogen oxides (5,6). Although a wide variety of nitro compounds found in the atmosphere were considered as direct-acting mutagens and/or carcinogens by the Ames Salmonella assays and in mammalian assay systems (7,8), the sum of the mutagenicity of the known compounds only accounts for 10–20% of the total mutagenicity of the atmospheric samples. It is suspected, therefore, that several unknown mutagenic compounds remain unidentified (9). These unknown mutagens are speculated to be present in the polar fractions of the extracts of the atmospheric samples (10,11).

In a previous study, we identified a potent mutagen, 3-nitrobenzanthrone (3-NBA), in the polar fraction of diesel exhaust particulates (see Figure 1 for chemical structures of 3-NBA and other related NBA studied in this article) (12). Now, 3-NBA is considered as a new powerful bacterial mutagen and clastogen, which is universally present in an atmospheric environment (13,14). It has been further reported that in vivo, 3-NBA is reductively activated to attack dG and dA in the DNA to form corresponding DNA adducts and also to induce lung cancer in F344 rats by oral administration (15,16).

Other studies have shown that a 2-nitro derivative of benzanthrone (BA), 2-nitrobenzanthrone (2-NBA), was also detected in an atmospheric environment (13,14). The evidence indicated that 2-NBA was formed due to the atmospheric nitration of the parental compound BA because only negligible amounts of 2-NBA were present in the original emission materials such as diesel exhaust particulates. Although the mutagenicities of 2-NBA in the Ames Salmonella assay have not yet been published, the compound was found to induce DNA damage in mammalian cell systems (17).

With regards to the nitro isomers of BA, we have already reported the mutagenic activities of 3-NBA and four other nitro isomers [9-nitro-, 11-nitro-, 3,9-dinitro- and 3,11-dinitro-BA (9-NBA, 11-NBA, 3,9-DNBA and 3,11-DNBA)] by the Ames assay. It was found that the latter four nitro derivatives were mutagenic; however, their activities were lower than that of the 3-nitro isomer (12). Surprisingly, the mutagenicities of these isomers were distributed in an extremely wide range (6–208 000 revertants/nmol to TA98 without the S9 mix) and clearly depended on the nitro substitution position of BA. In this study, in order to characterize the mutagenic activities of a series of nitro isomers of BA as well as to elucidate the underlying mechanisms of their mutagenic action, we developed four additional nitro isomers of BA [1-nitro-, 2-nitro-, 1,9-dinitro- and 3,9,11-trinitrobenzanthrone (1-NBA, 2-NBA, 1,9-DNBA and 3,9,11-TNBA)] and evaluated their mutagenicities by the Ames Salmonella assay along with the previously prepared NBAs using the following Salmonella typhimurium derivatives: TA98, TA100, YG1021 with an elevated nitro-eductase (N-rase) level, YG1024 with elevated O-acetyltransferases (OAT), TA98NR with a deficiency of N-rase and TA98/1,8-DNP6 deficient in OAT (8,18–20).

Moreover, the physicochemical properties [the lowest unoccupied molecular orbital (LUMO) energy, first reduction potential, hydrophobicity and orientation of nitro substituents]...
of the NBA isomers were also included in this study. Based on a large number of published works, nitroarenes generally require the metabolic reduction of a nitro substituent catalyzed by N-rase(s) to induce mutagenicity in the *Salmonella* strain (21). Therefore, the important features that affect the mutagenicity of nitroarenes (22–28) are as follows: (i) the ease of attacking the nitro groups with N-rase (dihedral angle of a nitro substituent to an aromatic ring plane) (29–33), (ii) the facilitation of the reduction of nitro groups [the first reduction potential ($E_{\text{red}}$) and LUMO energy] (22, 23, 34) and (iii) the smooth penetration of compounds into bacterial cells (hydrophobicity). An elucidation of the relationships between these physicochemical values and biological activities including mutagenicity and carcinogenicity may prove useful in predicting the human health risks of NBAs.

**Materials and methods**

**Chemicals**

Isomeric mono-, di- and tri-NBAs used in this study were shown in Figure 1. These samples were prepared from appropriately substituted nitrated methyl benzoate and nitrated iodonaphthalene via the reaction sequence of the Ullmann cross-coupling and the subsequent intramolecular Friedel–Crafts ring closure reaction. Details of the preparation as well as characterization are described elsewhere (35). Using this independent preparation method, possible contamination of other NBAs in each test sample is avoided. By the HPLC and GC/MS analyses, all the chemicals were verified to be $>$99.9% pure.

**Mutagenicity**

The *Salmonella typhimurium* strains TA98, TA98NR, TA98/1,8-DNP 6, TA100, YG1021 and YG1024 were used as the bacterial strains. The latter two strains were kindly provided by Dr T. Nomi of the National Institute of Health Sciences, Tokyo. The mutagenicity tests were carried out according to the method of Ames et al. (36, 37) with a slight modification, including a pre-incubation step (38). Ampicillin (25 mg/ml) and tetracycline (6.25 mg/ml) were added to the broth for the culture of YG1021 and YG1024. All mutagenicity assays were performed without S9 mix. Assays with an S9 mix metabolic activation system were also carried out with TA98, TA100, YG1021 and YG1024. An S9 mix containing 10% S9 was used as the metabolic activation system. S9, prepared from rat livers pre-treated with phenobarbital and 5,6-benzoflavone, was purchased from Kikkoman Co. (Chiba, Japan), and the cofactor was purchased from Oriental Yeast Co., Ltd (Tokyo). Furylfuramide (AF2) and benzo[a]pyrene (BaP) were used as positive controls for the TA98 and TA100 strains without and with the S9 mix, respectively, and 2-nitrofluorene was used for YG1021 and YG1024. Each sample was tested with a 20 min pre-incubation procedure for four doses. All the experiments were duplicated for each dose and repeated three times. Mutagenic activity was calculated from a linear portion of the dose–response curves and the averaged values of His$^+$ induced revertants/nmol of TA98 is assigned as 1.0.

**Table I. Mutagenicity of NBAs in strains possessing different metabolic capacities without S9 mix**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mutagenicity (revertants/nmol, –S9 mix)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>1-NBA</td>
<td>5560 (1.0)*</td>
</tr>
<tr>
<td>2-NBA</td>
<td>160 (1.0)</td>
</tr>
<tr>
<td>3-NBA</td>
<td>208 400 (1.0)</td>
</tr>
<tr>
<td>9-NBA</td>
<td>84 870 (1.0)</td>
</tr>
<tr>
<td>11-NBA</td>
<td>41 500 (1.0)</td>
</tr>
<tr>
<td>1,9-DNBA</td>
<td>46 560 (1.0)</td>
</tr>
<tr>
<td>3,9-DNBA</td>
<td>3530 (1.0)</td>
</tr>
<tr>
<td>3,11-DNBA</td>
<td>29 400 (1.0)</td>
</tr>
</tbody>
</table>

*The number in the parentheses indicates relative values when the number of His$^+$ induced revertants/nmol of TA98 is assigned as 1.0.*
The measurements of the octanol–water partition coefficient (K<sub>ow</sub>) performed by the RP-HPLC method, as described by Sarna et al., were made using a series of standard chemicals with published log<sub>10</sub> methanol:water (70:30) at a flow rate of 1.0 ml/min. A standard curve was prepared by a Shimadzu ODS-CLC(m) column (4.6 mm × 250 mm) using the calibration curve of standards.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mutagenicity (revertants/nmol, +S9 mix)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>1-NBA</td>
<td>39</td>
</tr>
<tr>
<td>2-NBA</td>
<td>153</td>
</tr>
<tr>
<td>3-NBA</td>
<td>1114</td>
</tr>
<tr>
<td>9-NBA</td>
<td>1010</td>
</tr>
<tr>
<td>11-NBA</td>
<td>–</td>
</tr>
<tr>
<td>1,9-DNBA</td>
<td>200</td>
</tr>
<tr>
<td>3,9-DNBA</td>
<td>956</td>
</tr>
<tr>
<td>3,11-DNBA</td>
<td>21</td>
</tr>
<tr>
<td>3,9,11-TNBA</td>
<td>109</td>
</tr>
</tbody>
</table>

Table II. Mutagenicity of NBAs in various Salmonella strains with S9 mix

TA98 series; 10, 5, 2, 1.25 ng/plate for TA100; 2, 1, 0.5, 0.25 ng/plate for YG1021; 0.05, 0.025, 0.0125, 0.00625 ng/plate for YG1024; 9-NBA: 5, 2.5, 1.25, 0.625 ng/plate for the TA98 series; 100, 50, 25, 12.5 for TA100; 10, 5, 2.5, 1.25 for YG1021; 1, 0.5, 0.25, 0.125 ng/plate for YG1024; 11-NBA: 10000, 5000, 2500, 1250, 625 ng/plate for YG1024, 1.9-DNBA: 10, 5, 2.5, 1.25 ng/plate for TA98 series; 100, 50, 25, 12.5 ng/plate for TA100; 10, 5, 2.5, 1.25 ng/plate for YG1021; 2, 1, 0.5, 0.25 ng/plate for YG1024, 3,9-DNBA: 10, 5, 2.5, 1.25 ng/plate for the TA98 series; 60, 30, 15, 10 ng/plate for TA100; 10, 5, 2.5, 1.25 ng/plate for YG1021; 2, 1, 0.5, 0.25 ng/plate for YG1024, 3,11-DNBA: 100, 50, 25, 12.5 ng/plate for the TA98 series; 100, 50, 25, 12.5, 6.25 ng/plate for YG1021; 30, 15, 7.5, 3.75 ng/plate for YG1024, 3,9,11-TNBA: 20, 29, 5, 2.5 ng/plate for TA98 series; 200, 1000, 500, 250 ng/plate for TA100; 50, 25, 12.5, 6.25 ng/plate for YG1021; 2, 1, 0.5, 0.25 ng/plate for YG1024.

Electrochemistry

<sub>E</sub><sub>red</sub> was measured with a bioanalytical system (BAS 50W electrochemical analyzer) by cyclic voltammetry analysis (CV) with a platinum working electrode at the rate of 100 mV/s. The chemicals (0.1 mmol) were dissolved in 10 ml of 0.1 M tetrabutylammonium hexafluorophosphate/acetonitrile solution.

Measurement of octanol–water partition coefficient (K<sub>ow</sub>)

The measurements of the octanol–water partition coefficient (K<sub>ow</sub>) were performed by the RP-HPLC method, as described by Sarna et al. (39). The HPLC system consisted of a Shimadzu LC-10A system with a Shimadzu LC10AD pump, Shimadzu SPD-10A UV detector set at 254 nm, and a Shimadzu ODS-CLC(m) column (4.6 mm × 250 mm) with a mobile phase of methanol:water (70:30) at a flow rate of 1.0 ml/min. A standard curve was prepared by a series of standard chemicals with published log<sub>10</sub> values in the range 0.95–6.00. The log<sub>10</sub>K<sub>ow</sub> values of NBAs were obtained from the retention volume using the calibration curve of standards.

Quantum data

The LUMO energy levels and dihedral angles between the nitro groups and the aromatic ring plane of NBAs were calculated by means of the semiempirical quantum mechanical method AM-1 using MOPAC ver.6.3. The initial geometries were constructed from the standard bond length and angles. Then, the geometry was completely optimized using algorithms in the MOPAC program.

Results

Mutagenicity

The parental compound BA was not found to be mutagenic at the tested doses in a test with all the Salmonella strains (40). The compound was reported to become mutagenic only after 60Co gamma irradiation (41). The mutagenic activity of NBA isomers was evaluated by using the lowest effective dose and the slope value (revertants/nmol). All the NBAs tested in this study were mutagenic in all the strains with and without the S9 mix (Table I). The mutagenic activity of 11-NBA was obtained from a clear dose-dependent positive response in the strains without the S9 mix; however, it was not obtained with the S9 mix because of the killing effect in the lowest effective dose that induced more than double the induction over the spontaneous revertants. With the TA98 strain, the most powerful mutagenic compound was 3-NBA, followed by 9-NBA, 1,9-DNBA, 3,9-DNBA, and 3,9,11-TNBA, suggesting that these NBAs induced a frameshift-type mutation. All the mutagenic activities of NBAs in TA100, which detects mutagens that cause base pair substitutions, were low when compared with TA98, with the exceptions of 1- and 2-NBA, both of which had the same level of mutagenic activity as that in TA98. The order of mutagenicity of NBA in TA98 without the S9 mix was 3-NBA > 9-NBA > 3,9-DNBA > 1,9-DNBA > 3,9,11-TNBA > 1-NBA > 3,11-NBA > 2-NBA. Generally, the mutagenicity of dinitro compounds is greater than that of their mononitro counterparts marked with nitropyrenes (42). However, this tendency was not observed in the NBA series; mononitro isomers exhibited the most potent mutagenic activity. In the case of NBAs, the mutagenic activity markedly depended on the location of the nitro group.

In order to further characterize the mutagenicity of NBA isomers, their activities were tested in the strains YG1021 and YG1024, which are a derivative of TA98 with elevated N-rase levels and an OAT-overproducing derivative of TA98, respectively, and TA98NR and TA98/1,8-DNP<sub>6</sub>, which are nitroreductase-deficient and acetyltransferase-deficient strains, respectively. The mutagenic potencies of 2-, 3- and 9-NBA were reduced in TA98NR when compared with TA98, suggesting that these were activated primarily by N-rases in TA98; however, 1-NBA exhibited a residual activity of only 94%. The mutagenic potencies of 1,9-DNBA and 3,9,11-TNBA were reduced in TA98NR, but those of 3,9-DNBA was not reduced.

The mutagenicities of 2- and 3-NBA were more efficiently detected using YG1021 and/or YG1024. The detection of the mutagenicity of 2-NBA was ~19 times more efficient by using YG1024; in comparison, 2-NBA exhibited almost the same level of mutagenicity to the OAT-deficient strain TA98/18-DNP<sub>6</sub> and was detected ~11 times more efficiently in YG1021 than that in TA98. 3-NBA exhibited an increased mutagenicity in TA98/1,8-DNP<sub>6</sub> however, it was not observed in the nitroreductase-deficient strain TA98NR. The mutagenicity of 3-NBA in YG1024 was ~30 times greater than that in TA98. The mutagenicities of 1-NBA, 3,9-DNBA and 3,11-DNBA were four times greater than that of the OAT-overproducing strain YG1024; however, they did not exhibit a reduced mutagenicity to TA98/1,8-DNP<sub>6</sub> when compared with TA98.

2-NBA was the only compound that displayed both reduced mutagenicity in TA98NR and increased mutagenicity in the nitroreductase-overproducing strain YG1021. All NBA exhibited the same level of mutagenicity or displayed a reduced mutagenicity in TA98/1,8-DNP<sub>6</sub> and exhibited a 4–30 times greater mutagenicity in YG1024 as compared to that in TA98.

<sub>E</sub><sub>red</sub> and LUMO energy of NBAs

The first reduction potential of NBAs measured using the CV system ranged from −0.7 V to −1.2 V (Table III). The calculated LUMO energy values ranged from −1.7 eV to −2.9 eV. A significant negative correlation was observed between the LUMO energy and <sub>E</sub><sub>red</sub> of the NBAs. On the basis of the chemical structure of the NBAs, the nitro substituents as well as the carbonyl group at the 7 position can be estimated to be chemically reduced. However, in general, the keto group in NBAs is less reductive than the nitro substituent. In fact, chemical reduction systems such as hydrazine
hydrate/palladium carbon, sodium sulphide and Sn/HCl reduce only the nitro substitution of NBA, and the keto group at the 7 position is inert (43,44). Thus, the first reduction potential ($E_{\text{red}}$) in Table III shows the reduction of nitro substituents and not the keto group.

The LUMO energy of the MNBAs varied with the nitration position and ranged from $-1.72$ eV to $-2.09$ eV. Among the MNBAs, 3-NBA exhibited the lowest value and 2-NBA, the highest. With an increase in nitration, the LUMO energy decreased in the order MNBA > DNBA > TNBA. This order can be easily estimated by considering that the introduction of the electron-withdrawing group NO$_2$ facilitates the reduction of compounds.

### Dihedral angle of nitro groups in relation to the aromatic ring plane

The dihedral angles of the nitro groups to the aromatic ring plane were calculated by MOPAC, and they are listed in Table III. Their values at the 1 and 11 positions of the nitro group in 1-NBA, 11-NBA, 1,9-DNBA, 3,11-DNBA and 3,9,11-TNBA were found to be $\sim$60° due to the significant interaction of peri hydrogen in the bay region (45). Nitro groups attached at the 2 and 9 positions were almost parallel to the aromatic ring. The nitro substituent at the 3 position was also found to be parallel to the aromatic ring for 3-NBA; however, it was not perfectly parallel in 3,11-DNBA and 3,9,11-TNBA.

### Hydrophobicity

Hydrophobicity is expressed by the octanol–water partition coefficient ($K_{\text{ow}}$) calculated from the chromatograph data. $K_{\text{ow}}$ obtained from the chromatograph data is listed in Table III. In this NBA series, the partition coefficients were fairly similar to each other within the range 3.60–3.99. The 2-nitro derivative was found to be the most hydrophobic and 1-nitro derivative to be the most hydrophilic; however, the difference in the values was within 0.39. This indicates that the penetration by NBAs into the cell surfaces is estimated to be nearly equal, and it is not a critical determinant for their mutagenicity.

### Discussion

In this study, we have analysed the mutagenicities of nine types of NBAs by using a variety of Salmonella tester strains. Among these compounds, 3-NBA was found to be the most potent mutagen in all the strains without an S9 mix. The metabolic activation studies on 3-NBA showed that the reduction of the nitro group to hydroxylamine was a critical step for inducing mutation. In a mammalian cell system, xanthine oxidase or NADPH was selected to induce nitro reduction of 3-NBA (15,16). In TA98NR, a variant of TA98 without N-rase, the number of revertant colonies induced by 3-NBA decreased, clearly indicating that mutagenicity was induced due to nitro reduction. This activation step would be necessary for all NBAs because all the compounds exhibited increased mutagenicities in YG1024; YG1024 increases the enzymes that induce the O-acetylation of hydroxylamines. All the NBAs also exhibited a decreased mutagenicity in the TA98NR strain, except for 1-NBA, 3,9-DNBA and 3,11-DNBA. These three compounds exhibit similar trends in mutagenicity and are unaffected by the lack of N-rase (TA98NR) and acetyltransferase (TA98/1,8-DPN$_6$). The reason for this discrepancy from the other NBAs remains unresolved; however, it is highly possible that the other enzymes can activate these compounds because it was well known that only a ‘classical nitroreductase’, which is one of the two nitroreductases of the S. typhimurium strain TA98, was deleted in S. typhimurium strain TA98NR (46–48).

From the studies of the LUMO energy and $E_{\text{red}}$ analysis of NBAs, the LUMO energy values of the MNBAs were considerably lower than that of mononitro polyaromatic hydrocarbons (PAHs) such as mononitropyrenes or mononitrofluoranthene ($\sim$−1.2 to −1.6 eV) and comparable to that of dinitroPAHs such as dinitropyrenes (−2.3 eV) (12). Therefore, it is estimated that the nitro groups of the MNBAs are more easily reduced than those of mononitroPAHs and as easily as dinitroPAHs. This facile reduction of the nitro substituent is due to the electron-drawing effect of the keto moiety at the 7 position of the NBAs. It is well known that the ease in the reduction of nitro substituents of chemicals causes a higher direct-acting mutagenic activity (25). This implies that aromatic compounds containing keto groups, when nitrated, may be potentially powerful direct-acting mutagens. Among the MNBAs, 3-NBA has the lowest LUMO energy (highest $E_{\text{red}}$) with the most potent mutagenic activity. The mutagenicity of the mononitro series of BAs were well correlated to the LUMO energy (or $E_{\text{red}}$), as described in the following equation: logTA98 = −1.23LUMO − 2.01 ($R^2 = 0.74$).

Further, the positions of nitro substitutions determine the ease in the reduction of the nitro groups. The nitro groups attached at the bay region of the parental BA tend to adopt a perpendicular orientation in relation to the aromatic ring and have higher LUMO energies, thereby resulting in lower mutagenicities. It is well known that nitroPAH with the nitro group along a perpendicular orientation exhibits either very weak or no direct-acting mutagenicity in Salmonella typhimurium TA98 (30). Among the NBAs with nonparallel
nitro groups, 11-NBA is an extremely weak mutagen, whereas 1-NBA exhibits moderate mutagenicity. This discrepancy could be explained by the ease in nitro reduction. Fu et al. (30) showed that, although a nitro substituent is oriented in a perpendicular direction, if the compound has a relatively low reduction potential, it may be a direct-acting mutagen. The report showed that even a slight decrease in \( E_{\text{red}} \) significantly increased the mutagenicity of compounds. The \( E_{\text{red}} \) value of 7-hydroxy-6-nitrobenz[a]pyrene and 3-acetoxy-6-nitroBaP was \(-0.86 \) V and \(-0.73 \) V, respectively. The former is a weak mutagen (31 revertants/nmol), whereas the latter is a moderately powerful mutagen (295 revertants/nmol); however, both have nitro substituents with a perpendicular orientation.

DNBA and TNBA, which have a lower reduction potential than that of MNBA, unexpectedly exhibited lower mutagenicity than the corresponding parental NBAs. The dependency of mutagenic activities on \( E_{\text{red}} \) (or the LUMO energy) that appeared in the mononitro series was not distinctly observed in the case of polynitrated BAs. 3,11-DNBA exhibited a higher mutagenic activity than 11-NBA, but lower than that of 3-NBA. 3,9-DNBA was also shown to have potent mutagenicity; however, its values were smaller than that of the parental 3-NBA and 9-NBA. The addition of a strong electron-withdrawing group such as NO2 to MNBAs decreases the LUMO values and facilitates nitro reduction, which would be expected to increase the mutagenicities. However, the mutagenicity of all polynitrated BAs was lower than that of 3-NBA. This tendency was also observed in the case of an increase in the nitration from dinitropyrenes to trinitropyrenes, the latter exhibiting lower mutagenic activities than the former. These retarding effects on the mutagenicity due to an increase in nitration were not sufficiently explained by the hydrophobicity factors because the hydrophobicities of the compounds were not significantly different. In order to induce the mutagenicity of nitroarenes, it is well known that the dominant metabolic pathway is the nitro reduction of the compounds. In general, nitro compounds were reductively activated to hydroxylamines followed by O-esterification, thereby forming electrophilic reactive nitrenium ions. However, when the compound exhibited sufficiently high \( E_{\text{red}} \) (or low LUMO energy), it was easy to estimate that the resulting hydroxyamino group was further reduced to the amino group efficiently; this yielded small amounts of hydroxymethylbenzanthrone as the ultimate mutagen. This might result in the saturation of mutagenicity as a function of LUMO energy. The following hypothesis is also confirmed: the mutagenicity of most of the NBAs in YG1021—a nitroreductase-overproducing strain—decreased as compared to those in TA98. The overproduction of N-rase may further enhance the reduction of hydroxylamines. 2-NBA was the only exception to this hypothesis because 2-NBA exhibited the highest value of \( E_{\text{red}} \) among all the NBAs tested in this study.

Among all the NBAs tested, 3-NBA exhibited the most mutagenic activity; this was due to not only a lower reduction potential but having the nitro group in a perfectly parallel orientation to the aromatic ring. Other factors such as the stability of hydroxylamine or the reactivity of nitrenium ions might affect the mutagenicity (48,49). In particular, an increase in the mutagenicity of YG1024, which is an acetyltransferase-duplicated strain, exhibits O-acetylation; further, the formation of subsequent nitrenium ions are of great importance in order to determine the mutagenic activity of NBAs.

In summary, we have shown in this study that (i) the mutagenicity of mono-nitrated BA clearly depend on the reduction potential and geometry of nitro groups attached to benzanthrone; (ii) each polynitrated derivative of BA showed high mutagenicity in TA98, but their activities were not dependent on the reduction potential; (iii) on the basis of the reduction potential, aromatic nitroketones might be potentially mutagenic so the environmental nitration of polyaromatic keto compounds is fairly hazardous to humans. Since a subsequent study on the nitration of aromatic keto compounds and their mutagenicities would be important, this study is currently being conducted by our group.

References


