Formation of C8-modified deoxyguanosine and C8-modified deoxyadenosine as major DNA adducts from 2-nitropyrene metabolism mediated by rat and mouse liver microsomes and cytosols

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2-Nitropyrene, the geometric isomer of the most studied nitro-polycyclic aromatic hydrocarbon (nitro-PAH), 1-nitropyrene, is an environmental contaminant detected in ambient air and a potent direct-acting mutagen. Its metabolic activation leading to the formation of DNA adducts was studied. The activated metabolite, N-hydroxy-2-aminopyrene, was prepared and reacted with calf thymus DNA. Upon enzymatic hydrolysis of the DNA, the resulting nucleosides were separated by HPLC, and the adducts were characterized by mass and proton NMR spectral analysis. Both N-(deoxyguanosin-8-yl)-2-aminopyrene and N-(deoxyadenosin-8-yl)-2-aminopyrene, in a 5:2 ratio, were identified. These adducts were then utilized as standards to identify the DNA adducts formed from reaction of [3H]2-nitropyrene with DNA mediated by liver microsomes and cytosomes of mouse and rat. In all cases, both adducts were formed. The quantities of the two adducts formed in each system were: mouse liver microsomes (11.3 pmol [3H]2-nitropyrene/mg DNA), rat liver microsomes (23), mouse liver cytosol (11.4) and rat liver cytosol (5.1). Thus, these adducts were formed in highest yield from rat liver microsomes and the lowest from rat liver cytosol. The deoxyguanosine/deoxyadenosine adduct ratio was higher from rat and mouse liver microsomes (7.8:9.2) than from rat and mouse liver cytosols (2.5:3.1). Our results represent the first direct demonstration of a C8-deoxyadenosine adduct being formed as a major product from the reaction of a nitro-PAH metabolite with DNA.

Introduction
Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are widespread genotoxic environmental pollutants (1-6) that may pose a risk to human health. Nitro-PAHs require metabolic activation and evidence has shown that reduction of the nitro functional group (nitroreduction) and ring-oxidation followed by nitroreduction are the most important activation pathways in both the bacterial and mammalian systems (3-22). We have been interested in the metabolic activation of nitro-PAHs and their structure-activity relationships, and have studied a series of structurally related nitro-PAHs including 2- and 9-nitroanthracene (23,24), 1-, 3- and 6-nitrobenzo[a]pyrene (14-16,25), 1- and 3-nitrobenzo[e]pyrene (6,21), 4-nitropyrene (6) and 7-nitrobenz[a]anthracene (26).

1-Nitropyrene is the most abundant environmental nitro-PAH and its carcinogenic activity has been studied more closely than that of other nitro-PAHs (1-7, 10-13, 27-31). Thus, in connection with the study of structure-activity relationships, it is important to study its structural isomers, 2- and 4-nitropyrene. 2-Nitropyrene has recently been found in ambient particulate organic matter in quantities comparable to 1-nitropyrene (32,33). Unlike 1-nitropyrene, which is formed during combustion processes by nitration of pyrene via an ionic mechanism, 2-nitropyrene is formed via a free radical mechanism. This is thought to occur during source-receptor transport by atmospheric reactions of adsorbed or gas-phase pyrene with oxides of nitrogen, nitric acid and other related species (33). 2-Nitropyrene has been found to exhibit much higher direct-acting mutagenicity (2225-2569 revertants/nmol) than 1-nitropyrene (~470 revertants/nmol) (1,4,34,35). Consequently, it is essential to study its metabolic activation and compare these results with those obtained with 1-nitropyrene. Its potent direct-acting mutagenicity implicates nitroreduction of 2-nitropyrene as an important metabolic activation pathway for mutation. In this study, we report the preparation of N-hydroxy-2-aminopyrene, its reaction with calf thymus DNA for the synthesis of DNA adducts, and utilization of these adducts as standards for the binding of 2-nitropyrene with DNA mediated with rat and mouse liver microsomes and cytosomes. Two adducts, N-(deoxyguanosin-8-yl)-2-aminopyrene and N-(deoxyadenosin-8-yl)-2-aminopyrene, are formed in substantial quantities in all four enzyme systems. Our results present the first direct example of the formation of a C8-deoxyadenosinyl adduct as a major product of the reaction of nitro-PAHs with DNA. Since the carcinogen-deoxyadenosine adducts have been suggested as being important for the genotoxic activity of nitro-PAHs (36,37), our findings may be valuable in the elucidation of the potent direct-acting mutagenicity of this compound.

Materials and methods

Materials
2-Nitropyrene was synthesized via nitration of 3,4,9,10-tetrahydropyrene followed by aromatization with 2,3-dichloro-5,6-dicyano-1,4-benzoquione. [G-3H2]-Nitropyrene (sp. act., 597 mCi/mmol) was prepared by catalytic tritium exchange with 2-nitropyrene at the Chemsyn Science Laboratories, Lenexa, KS. Liver microsomes and cytosol of male Sprague-Dawley rats (150-200 g body wt, obtained from the NCTR breeding colony) were prepared from the general method of Westra for the synthesis of DNA adducts, by ultracentrifugation with the exception that the livers were perfused with 0.15 M KCl before removal from the body (38). Mouse liver microsomes and cytosol were prepared from 86C3F1 mice (obtained from the NCTR breeding colony) in a similar manner, except that the livers were not perfused. Protein concentrations were determined by the Lowry method (39).

Preparation of N-hydroxy-2-aminopyrene
 Conversion of 2-nitropyrene to N-hydroxy-2-aminopyrene was accomplished by the general method of Westra for the synthesis of N-hydroxyamino-PAHs from the corresponding nitro-PAHs (40). 2-Nitropyrene (25 mg) in tetrahydrofuran (THF, 25 ml) under argon was cooled to 2°C with an ice bath and 3 mg of 10% Pd on carbon was added with stirring. As soon as the catalyst was fully dispersed, 30 μl of 90% hydrazine was added. After 1 h, the reduction was terminated and...
The n-butanol phase was taken to dryness under reduced pressure and redissolved in 0.1 mM EDTA, pH 7.1. The concentration of the DNA solution was determined spectrophotometrically. A 20 mg (85.8 nmol) dose of 2-aminopyrene was added to 20 ml of argon-purged ethanol and the reaction mixture was filtered through Celite at reduced pressure under argon. The residue was washed twice with 25 ml of THF. The filtrates were combined and the solvent reduced under reduced pressure. After removing a sample for NMR analysis, the sample was flooded with argon and stored at -5°C. The structure was determined by analysis of its NMR (Table I) and mass spectral data. The NMR spectrum in DMSO-d$_6$ showed the presence of a trace amount of 2-aminopyrene, as exhibited by the appearance of a singlet at 7.42 p.p.m.

### Table I. Proton magnetic resonance spectral data of N-hydroxy-2-aminopyrene and of the DNA adducts formed from reaction of N-hydroxy-2-aminopyrene with calf thymus DNA

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Coupling constant (Hz)</th>
<th>Chemical shift (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adduct I*</td>
<td></td>
<td>Adduct II*</td>
</tr>
<tr>
<td>H-1, H-3</td>
<td></td>
<td>8.52</td>
</tr>
<tr>
<td>H-4, H-10</td>
<td></td>
<td>8.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>H-5, H-9</td>
<td></td>
<td>8.02&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>H-6, H-8</td>
<td></td>
<td>8.13</td>
</tr>
<tr>
<td>H-7</td>
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<td>7.91</td>
</tr>
<tr>
<td>H-1'</td>
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<td>6.54</td>
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<tr>
<td>H-2'</td>
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<tr>
<td>H-3'</td>
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<tr>
<td>H-4'</td>
<td></td>
<td>4.08</td>
</tr>
<tr>
<td>H-5'</td>
<td>4.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>H-5*</td>
<td>3.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>H-2</td>
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<td></td>
</tr>
</tbody>
</table>

*In methanol-d$_4$.

<sup>a</sup>In DMSO.

<sup>b</sup>-Assignments may be reversed.

the reaction mixture was filtered through Celite at reduced pressure under argon. The residue was washed twice with 25 ml of THF. The filtrates were combined and the solvent reduced under reduced pressure. After removing a sample for NMR analysis, the sample was flooded with argon and stored at -5°C. The structure was determined by analysis of its NMR (Table I) and mass spectral data. The NMR spectrum in DMSO-d$_6$ showed the presence of a trace amount of 2-aminopyrene, as exhibited by the appearance of a singlet at 7.42 p.p.m.

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<tbody>
<tr>
<td>Adduct I*</td>
<td>Adduct II*</td>
<td>N-Hydroxy-2-aminopyrene&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>H-1, H-3</td>
<td>8.52</td>
<td>9.2</td>
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<tr>
<td>H-4, H-10</td>
<td>8.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.2</td>
</tr>
<tr>
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</tr>
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</tr>
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<td>H-2'</td>
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<td>H-5*</td>
<td>3.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.0</td>
</tr>
<tr>
<td>H-2</td>
<td>8.08</td>
<td>7.7</td>
</tr>
</tbody>
</table>

*In methanol-d$_4$.

<sup>a</sup>Assignments may be reversed.
**Results**

Reaction of N-hydroxy-2-aminopyrene with calf thymus DNA and characterization of adducts

N-Hydroxy-2-aminopyrene, freshly prepared from reduction of 2-nitropyrene with hydrazine catalyzed by Pd/C, was incubated with a 10-fold excess (by wt) of calf thymus DNA. The DNA fraction precipitated by ethanol was hydrolyzed enzymatically. The resulting nucleoside adducts were separated by reversed-phase HPLC (Figure 1). Each chromatographic peak was first monitored by analysis of its UV-visible absorption spectral data, and if necessary, by analysis of mass spectral data as well. The chromatographic peak eluted at 19.2 min did not contain any adduct. The chromatographic peaks eluted at 25.2 and 28.0 min had UV-visible absorption spectra indicating a pyrenyl chromophore (Figure 2), suggesting that they were nucleoside adducts.

When dissolved in methanol or methanol/water for FAB mass spectral measurements using thioglycerol, the material (adduct I) eluting at 25.2 min (Figure 1) did not show significant signal levels. However, satisfactory spectra were obtained using DMSO as solvent when the resulting solution was added to thioglycerol matrix and mixed directly on the FAB probe tip. As shown in Figure 3A, this adduct showed the characteristic ions at m/z 483 assigned as the [M+Na]⁺ ion, at m/z 505 assigned as the [M+2Na]²⁺ ion, and at m/z 483 assigned as the [M+H]⁺ ion. Loss of 116 daltons (deoxyribose) from the protonated molecule gave the expected BH₂⁺ ion at m/z 367. The weaker ion at m/z 389 was assigned to the characteristic [BHNa]⁺ ion. The relatively lower intensity of this ion is attributed to smaller propensity for fragmentation from the [M+Na]⁺ ion. Thus, the FAB mass spectral data suggested that adduct I was a deoxyguanosinyl-aminopyrene adduct.

In order to establish that the mol. wt for adduct I was 482, and also to show unambiguously that no other adducts were present in this sample, an additional experiment was performed. A constant neutral loss scan (FAB/MS/MS) was employed to identify all parent ions showing the nucleoside-characteristic loss of 116 daltons upon collisionally activated decomposition (42). This technique allows nucleosides to be readily identified in mixtures or in the presence of the intense FAB matrix background signals, since the loss of 116 daltons is not observed from typical FAB matrix ions and is not particularly prominent from non-nucleosides. The FAB/MS/MS, constant neutral loss scan for adduct I is shown in Figure 4. The prominent signals at 483, 505 and 527 daltons correspond to the protonated molecule and the corresponding mono- and disodium containing ions observed.
Fig. 3. FAB mass spectra of (A) \( N\)-\( \text{deoxyguanosin-8-yl}\)-2-aminopyrene and (B) \( N\)-\( \text{deoxyadenosin-8-yl}\)-2-aminopyrene. See Materials and methods for experimental conditions.

Fig. 4. Constant neutral loss scan for adduct I showing those ions from the FAB mass spectrum which lose 116 daltons upon collisional activation.

in the FAB/MS spectrum. Note that no other ions corresponding to adducts from other likely aminopyrene--nucleoside adduct ions are observed. The only other ion showing a loss of 116 daltons is much lower in mass, at \( m/z \) 376, and is also considerably less intense. This ion \((m/z 376)\) corresponds to a cluster ion formed via the associations of a thioglycerol (the FAB matrix) and unmodified deoxyguanosine. These data indicate conclusively that the only aminopyrene modified nucleoside in adduct I has a mol. wt of 482 daltons. This sample also appears to contain a small amount of unmodified deoxyguanosine.

The structure was further characterized based on analysis of the 500 MHz proton NMR spectrum (Figure 5A and Table I).

The resonances at 2.22–6.54 p.p.m. were characteristic of a 2'-deoxyribose ring (43), which showed that this was indeed a modified mononucleoside adduct derived from DNA (Table I). Likewise, all the C–H resonances from the pyrene moiety (7.91–8.52 p.p.m.) were present, except for the 2 position, which established that this adduct was substituted at \( N_2 \) of the pyrene ring. There were no additional resonance besides those which are attributed to the deoxyribose or pyrene moieties, which was consistent with a substitution on a nucleic acid base with no remaining CH proton. This indicated a C8-deoxyguanosinyl adduct. Support for this conclusion was found by a broad exchangeable resonance at 6.49 p.p.m. which was characteristic of a guanine NH2 resonance (44). Thus, it was concluded that adduct I was \( N\)-\( \text{deoxyguanosin-8-yl}\)-2-aminopyrene. Other exchangeable resonances that might be expected were not seen due to rapid exchange in this particular sample preparation. The coupling constants associated with the 2'-deoxyribose ring of this adduct were similar to those previously reported for \( N\)-\( \text{deoxyguanosin-8-yl}\)-2-aminofluorene (43). The unique spectral parameters of this type of C8-amino substituted guanine adduct are large values of \( J_{1'-2'} \) and small values of \( J_{2' \gamma} \), which are indicative of a high population of C2'-endo sugar ring conformation and a predominant gauche+ \((\gamma = 60^\circ)\) conformation about the C4'–C5' bond. These are unusual preferred conformations that result from a stabilizing interaction between the 8-NH group and the sugar moiety (43,45). It provides further support of the structure as \( N\)-\( \text{deoxyguanosin-8-yl}\)-2-aminopyrene.

The proton NMR spectrum for the sample also shows the presence of a second set of resonances at \( \sim 20\% \) the height of the primary resonances. These minor resonances are most easily seen on the upfield side of the 1' and 3' regions (Figure 5A). Attempts to isolate this minor product by employing several different HPLC conditions were unsuccessful. However, Roy et
Formation of DNA adducts

Table II. Binding of \[^{3}H\]2-nitropyrene to calf thymus DNA mediated by mouse and rat liver microsomes and cytosols

<table>
<thead>
<tr>
<th>Enzymatic system</th>
<th>[^{3}H]2-nitropyrene(^b) (pmol)</th>
<th>Adduct (^{10}) nucleotides</th>
<th>dGuo(^b)</th>
<th>dAdo(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse liver microsomes</td>
<td>11.3 ± 3.5</td>
<td>3.6</td>
<td>7.8 ± 2.2</td>
<td>3.1 ± 1.8</td>
</tr>
<tr>
<td>Mouse liver cytosol</td>
<td>11.4 ± 2.2</td>
<td>3.7</td>
<td>9.2 ± 2.6</td>
<td>2.5 ± 1.1</td>
</tr>
<tr>
<td>Rat liver microsomes</td>
<td>23.0 ± 4.5</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat liver cytosol</td>
<td>5.1 ± 1.2</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^b\)In a 2 ml incubation, 40 nmol/ml of \[^{3}H\]2-nitropyrene (sp. act., 597 mCi/mmol) was incubated hypoxically with the mouse and rat liver microsomes and cytosols in the presence of 2 mg of calf thymus DNA at 37°C for 4 h. See Materials and methods for detailed incubation and work-up conditions.

\(^b\)Averaged data from three experiments.

al. (46) reported that in vitro metabolic activation of 2-nitropyrene to DNA adducts mediated by xanthine oxidase generated the N-(deoxyguanosin-8-yl)-2-aminopyrene which was free from this minor product.

The FAB mass spectrum of the adduct (adduct II) eluting at 28 min (Figure 1) showed characteristic ions at \[^{m/z}\] 467, assigned as the \([M+H]^+\) ion and at \[^{m/z}\] 351 corresponding to the \([BH_2]^+\) ion. The base peak at \[^{m/z}\] 351 resulted from loss of 116 daltons (deoxyribose) from the protonated molecule. These results confirmed that this was a nucleoside adduct from DNA, and from the mol. wt. that this adduct was a N-(deoxyadenosinyl)-aminopyrene.

The 500 MHz proton NMR spectrum (Figure 5B) exhibited spectral parameters similar to those described above for adduct 1. Central to the identification of adduct II was the detection and assignment of a non-exchangeable singlet observed at 8.08 p.p.m. (Table I). Although buried in the aromatic region due to overlap with protons, 4, 5, 9 and 10 of the pyrenyl moiety (Figure 5A), it was initially located by the altered intensities from a simple AB coupling pattern that is best seen in expansions of this region. When recorded to DMSO, this singlet is clearly resolved (8.06 p.p.m.). It was assigned to H2 of adenine rather than H8 based on the abnormal conformation that is characteristic of the sugar moiety of this type of C8-substituted adduct (43,45). This was evident in the similarity in the coupling constants between adducts I and II (Table I). The chemical shift was comparable to that of 1-nitropyrene and it has a greater mutagenicity relative to 1-nitropyrene, we have been studying its metabolic activation leading to DNA adduct formation. Reaction of N-hydroxy-2-aminopyrene with 2-mg of calf thymus DNA at pH 5 resulted in the formation of both N-(deoxyguanosinyl)-2-aminopyrene pmol \[^{3}H\]2-nitropyrene/mg DNA, which is several fold higher than that of rat liver cytosol (Table II). Upon separation of the DNA adducts by reversed-phase HPLC and co-chromatography with the non-radioactive N-(deoxyguanosin-8-yl)-2-aminopyrene and N-(deoxyadenosin-8-yl)-2-aminopyrene adducts as internal standards, the formation of these two adducts was established and quantified. As an example, Figure 6 shows the reversed-phase HPLC profile for the separation of DNA adducts formed from incubation of \[^{3}H\]2-nitropyrene with mouse liver cytosol in the presence of calf thymus DNA. The results shown in Figure 6 clearly indicate that there may be a third adduct eluted at 31 min. Due to lack of an internal standard, its structure was not determined.

The deoxyguanosine/deoxyadenosine (dGuo/dAdo) adduct ratio was subsequently determined. As shown in Table II, both mouse and rat liver microsomes had similar dGuo/dAdo adduct ratio, with values of 8.3—9.7. On the other hand, mouse and liver cytosols resulted in the same dGuo/dAdo adduct ratio, 2.8 and 2.7, which is close to that from reaction of N-hydroxy-2-aminopyrene (~2.5).

Discussion

Since 2-nitropyrene has been identified as a ubiquitous environmental contaminant in ambient air at concentrations comparable to 1-nitropyrene and it has a greater mutagenicity relative to 1-nitropyrene, we have been studying its metabolic activation leading to DNA adduct formation. Reaction of N-hydroxy-2-aminopyrene in vitro with calf thymus DNA at pH 5 resulted in the formation of both N-(deoxyguanosin-
8-yl)-2-aminopyrene and \(N\)-(deoxyadenosin-8-yl)-2-aminopyrene in significant amounts. Similarly, in vitro incubations of \([^{3}H]2\)-nitropyrene with mouse liver microsomes, mouse liver cytoso1, rat liver microsomes and rat liver cytoso1 in the presence of calf thymus DNA under hypoxic conditions all resulted in the formation of both of these adducts as major products. Thus, the similarity of the results obtained from reactions of calf thymus DNA with \(N\)-hydroxy-2-aminopyrene and with the metabolite(s) of \([^{3}H]2\)-nitropyrene suggest that nitroreduction of 2-nitropyrene to \(N\)-hydroxy-2-aminopyrene is the metabolic activation pathway leading to the formation of these two DNA adducts. The yields of DNA adducts are higher in the in vitro incubations of \([^{3}H]2\)-nitropyrene with the rat liver microsomes than by the rat liver cytoso1 (Table II). It is known that the enzymes responsible for nitroreduction of nitro-PAHs contained in the hepatic microsomal fraction are mainly the reduced forms of the cytochrome P450 isozymes and NADPH-cytochrome P450 reductase. In the hepatic cytoso1 fraction, the major nitroreductases are aldehyde oxidase, DT-diaphorase and xanthine oxidase (1,3,5,6,11,31). Thus, our results, shown in Table II, indicate that the metabolic activation of 2-nitropyrene to the reactive species for covalent binding to DNA occurs via the nitroreduction pathway, and that the rat liver microsomal enzymes exhibit higher activity than the rat liver cytoso1 enzymes. These results are consistent with the previous findings that DNA binding of 1- and 3-nitrobenzo[a]pyrene, 7-nitrobenz[a]anthracene, 9-nitroanthracene and their transdihydriodols catalyzed by rat liver microsomes is several fold higher than that catalyzed by rat liver cytoso1 (2.5—10.1 pmol bound/mg DNA) (49).

It is interesting to point out that the dGuo/dAdo adduct ratio is higher from rat and mouse liver microsomes than from rat and mouse liver cytoso1s. Thus, the \(N\)-(deoxyadenosin-8-yl)-2-aminopyrene adduct is formed in a higher ratio from the rat and mouse liver cytoso1 systems than from the rat and mouse liver microsomal systems (Table II). Since, in all the cases, calf thymus DNA is the only source of DNA for binding, the different dGuo/dAdo adduct ratios formed from microsomal and cytoso1 fractions suggest that the active species derived from metabolic activation of 2-nitropyrene may be different between the microsomal and cytoso1 systems.

In vitro reactions of DNA with \(N\)-hydroxy arylamines, formed either from enzymatic oxidation of arylamines, from enzymatic reduction of nitro-PAHs and nitroso-PAHs, or from chemical synthesis, generally yield C8-deoxyguanosine adducts as the predominant products and N\(^{2}\)-deoxyguanosine adducts as minor products (1,4,6,11,18,47,48,50—53). C8-Modified deoxyadenosine adducts have only been reported as minor products (1—3% of the total DNA adducts (from the reactions of DNA with \(N\)-hydroxy-4-aminobiphenyl and \(N\)-hydroxy-3,2'-dimethyl-4-aminobiphenyl) in vivo and with 4-aminobiphenyl in vivo (47,48). Thus, formation of \(N\)-(deoxyadenosin-8-yl)-2-aminopyrene as a major product from reactions of calf thymus DNA with \([^{3}H]2\)-nitropyrene mediated by mouse and rat liver microsomes and cytoso1s is unexpected.

Massaro et al. (36) reported that several nitro-PAHs exhibited considerable direct-acting mutagenicity in \(S\)almonella tester strains TA96 and TA102 which have adenine—thymine base pairs at the mutational target, providing evidence that adenine adducts, similar to the guanine adducts, could also play an important role in the induction of mutation by these compounds. Further studies by theoretical quantum chemical calculations have also predicted that formation of C8-deoxyadenosine adducts derived from nitro-PAHs and arylamines is possible (37). Furthermore, based on the study of specificity of mutagenesis by 4-aminobiphenyl, Lasko et al. (54) have obtained evidence that \(N\)-deoxyadenosin-8-yl)-4-aminobiphenyl adduct was a premutational lesion. The \(N\)\(^{2}\)-deoxyadenosine adducts formed from several PAHs, including 7,12-dimethylbenz[a]anthracene and benzo[c]phenanthrene, have also been proposed to correlate with the tumor initiating activity of the parent compounds (55,56). Thus, formation of \(N\)-(deoxyadenosin-8-yl)-2-aminopyrene from \(N\)-hydroxy-2-aminopyrene or from \([^{3}H]2\)-nitropyrene at similar levels may be biologically very important, and consequently, further investigations are warranted.

Deliclos et al. (18) reported that reaction of \(N\)-hydroxy-6-aminochrysene with DNA resulted in the formation of \(N\)-(deoxyadenosin-8-yl)-6-aminochrysene accounting for 32% of the total DNA adduct formed. This adduct was proposed to be formed from facile oxidation of the \(N\)-(deoxyadenosin-8-yl)-6-aminochrysene either on the DNA strand and/or after isolation. If this is the case, it would be interesting to understand why only \(N\)-hydroxy-6-aminochrysene and \(N\)-hydroxy-2-aminopyrene produce C8-deoxyadenosine adducts in high yield, while all the other \(N\)-hydroxy-arylamines so far studied, including \(N\)-hydroxy-1-aminopyrene, either do not yield an adduct of this type or produce it only in a very low yield. It is also important to understand why \(N\)-(deoxyadenosin-8-yl)-2-aminopyrene, \(N\)-(deoxyadenosin-8-yl)-4-aminobiphenyl and \(N\)-(deoxyadenosin-8-yl)-3,2'-dimethyl-4-aminobiphenyl are stable (resistant to air oxidation), while \(N\)-(deoxyadenosin-8-yl)-6-aminochrysene is facilely oxidized to \(N\)-(deoxyinosin-8-yl)-6-aminochrysene.

Acknowledgements

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Formation of DNA adducts


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