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Short Communication

The Hydrolytic Stability of Hydroxypropano Adducts of 2′-Deoxyguanosine Formed by Reaction with Acrolein and Crotonaldehyde

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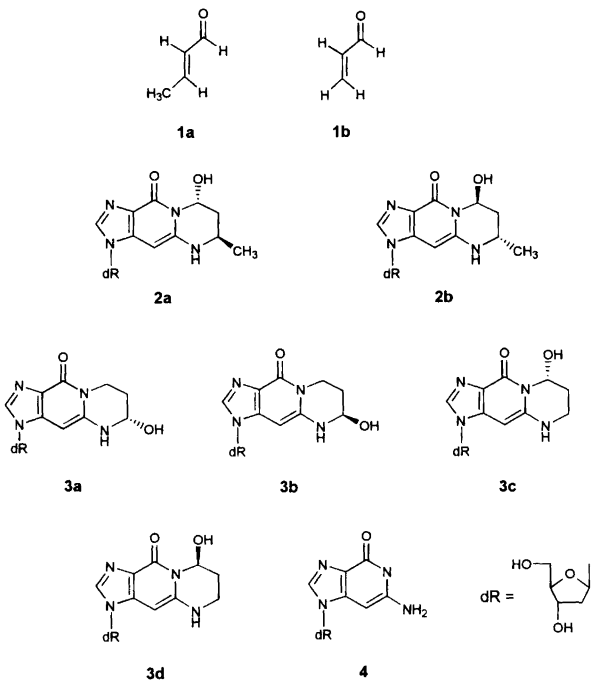
Hydroxypropano adducts of DNA are formed in living organisms by a reaction between the heterocyclic bases and α,β-unsaturated carbonyl compounds such as malonaldehydes, acrolein and crotonaldehyde.[1,2] α,β-Unsaturated carbonyl compounds are produced intracellularly as a result of oxidative stress induced by environment-related factors, such as an increased ozone level or an exposure to heavy metals or irradiation. Crotonaldehyde (**1a**) and acrolein (**1b**) can also be produced as metabolites of some chemotherapeutic agents or carcinogens, or introduced into the human body via a direct exposure to these compounds, widely used in the chemical industry. Hydroxypropano adducts of guanine have been shown to cause miscoding in DNA biosynthesis.[2] An increase in the level of these adducts in DNA can be considered a first indication of oxidative stress or exposure to mutagenic agents to a harmful extent. As the level of the modified bases is low, sensitive methods for analysis of these adducts in DNA are required.



There are two different basic methods of analysis of DNA adducts: direct analysis of intact DNA, or analysis of the nucleosides, nucleotides or bases after degradation of DNA.[3] The release of monomeric units can be achieved by using either chemical[4] or enzymatic cleavage,[3] and the analysis itself can be performed in several ways, including HPLC with fluorescent or electrochemical detection,[5] ^{32}P-post-labelling,[6] GC/ESI–MS,[7] GC/ID–MS[8] and HPLC/ESI–MS.[4]

An approach where the nucleoside bases are released by acid-catalysed hydrolysis of the *N*-glycosidic bonds and subsequently analysed by LC/ESI–MS/MS is being studied in our laboratory.[9] Particularly in this method, but also with any other analysis method, it is important to know the stability of the adducts, so as not to expose them to conditions under which they are not stable. The hydrolytic stability of the *N*-glycosidic bond of nucleosides is known to vary significantly depending on the structure of the base;[10] analysis of adducts which destabilise the *N*-glycosidic bond relative to natural nucleosides would be simplified as the modified bases could be released selectively by careful choice of conditions.

In this paper, we present a study of the stability of the hydroxypropano adducts **2a**, **2b** and **3a**–**3d** formed by a

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reaction of 2′-deoxyguanosine (**4**) with crotonaldehyde (**1a**) or acrolein (**1b**). The acid-catalysed reactions have been studied and the reactivity of the adducts is compared with that of 2′-deoxyguanosine, to facilitate the development of analysis methods of mutated DNA bases.

The 2′-deoxyguanosine adducts of acrolein and crotonaldehyde were prepared as described in the literature.[11,12] The reaction of crotonaldehyde with 2′-deoxyguanosine gave a diastereomeric mixture of 8-hydroxy-6-methyl-1,N^2-propanodeoxyguanosines **2a** and **2b**. The diastereomers were stable and were separated by RP-HPLC. No interconversion of the pure isomers was observed. The NMR spectra of the compounds synthesised were fully consistent with those reported before.[11] The reaction of **4** with acrolein has been reported[12] to yield two diastereomeric pairs, **3a**+**3b** and **3c**+**3d**. In our hands only **3a** and **3b**, 6-hydroxy-1,N^2-propanodeoxyguanosines, were obtained. **3a** and **3b** were in rapid equilibrium with each other, and were not separated, but studied as a mixture.

The reactions of **2a**, **2b**, **3a**+**3b** and **4** were studied under acidic conditions as a function of pH. The only reaction observed was depurination of the nucleosides. With all of the substrates, only one reaction product was detected, which in the case of 2′-deoxyguanosine was identified as guanine base, by spiking of the product with an authentic sample. The HPLC–MS analysis of the product obtained by the hydrolysis of a mixture of **2a** and **2b**, gave a molecular mass of 222.2 ($M+1$), consistent with the structure of a depurination product. With all the 2′-deoxyguanosine adducts studied, the UV spectra observed were fully consistent with those reported earlier for the hydroxypropano guanine adducts formed upon depurination.[11,12]

The logarithmic rate contants of depurination of the adducts **2a**,**b**, **3a**,**b** and **4** obtained at 60 °C, are shown as a function of pH in Fig. 1. It is seen that in every case the reaction shows a first-order dependence on hydronium ion concentration, consistent with previous reports on hydrolysis of purine nucleosides.[10] It is also seen that the reactivity differences between 2′-deoxyguanosine (**4**) and the adducts are very small: the maximum reactivity difference between **4** and the adducts is only a factor of 2–3, **4** being more reactive than the adducts. The small differences in the reactivity are most clearly shown by the second-order rate constants of the hydronium-ion-catalysed reaction shown in Table 1.

The results in Fig. 1 clearly show that the reaction is specific acid catalysed. To study whether there is a catalysis also by general acids, we took some measurements at a higher buffer concentration. No catalysis by the buffer was observed, instead the rate slightly decreased as the buffer concentration increased. Studies in more basic buffers showed that the base catalysed decomposition of the adducts is very slow, if it exists at all. Only in 0.01 M NaOH was a clearly detectable cleavage of the adducts observed. The half-lives of **2a** and **3a** were 21 and 64 h, respectively at 60 °C, while 2′-deoxyguanosine **4** appeared to be stable under these

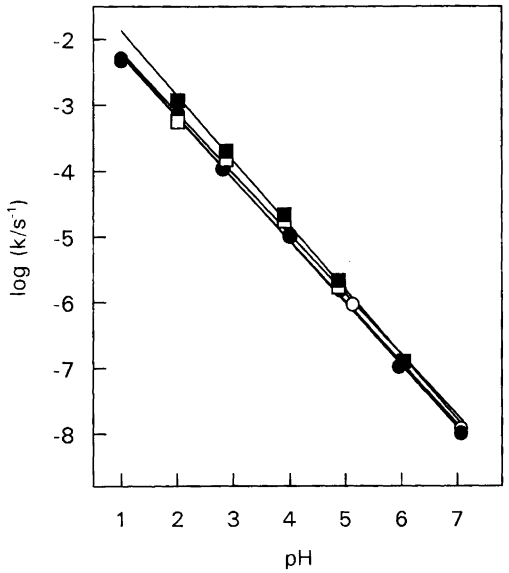

Fig. 1. Logarithmic first-order rate constants of the hydrolysis of 2′-deoxyguanosine hydroxypropano adducts as a function of pH at 60 °C. Notation: ○, **2a**; ●, **2b**; □, **3a**+**3b** and ■, **4**.

Table 1. Second-order rate constants of the hydronium-ion-catalysed depurination of 2′-deoxyguanosine and its hydroxypropano adducts at 60 °C.

Substrate	k_2/dm^3 s^{-1} mol^{-1}
2a	0.046±0.001
2b	0.050±0.001
3a+**b**	0.056±0.004
4	0.120±0.003

conditions. This reaction does not involve depurination, the products formed have clearly different retention times and UV spectra. Most probably the base structure is being destroyed under alkaline conditions.

The depurination generally proceeds through a unimolecular cleavage of a protonated substrate to yield a free base and a furanosyl oxocarbenium ion.[10] This mechanism is most probably utilised in the hydrolysis of hydroxypropano adducts as well. Consistent with this, the pH–rate profile of the depurination is linear within the pH range studied.

In conclusion, formation of a hydroxypropano adduct does not seem to destabilise the *N*-glycosidic bond of guanosine under acidic conditions, but the adducts studied undergo depurination approximately as fast as 2′-deoxyguanosine does. Under alkaline conditions the adducts may be slightly less stable than guanosine. The reaction observed under alkaline conditions probably results in degradation of the base moiety rather than in depurination.

Experimental

The synthesis of the hydroxypropano adducts has been reported before.[11,12] Hydrolysis reactions were studied

in 0.05 M buffer solutions, the pH of which was measured at 60 °C. The aliquots withdrawn were analysed by RP-HPLC (Hypersil ODS, 5 × 250 mm, 5 μm particle size). The eluent was a mixture of 0.05 M acetic acid buffer (pH 4.3) and acetonitrile (4–11% depending on the substrate). UV detection at 254 nm was employed. Rate constants were calculated by using an integrated rate-law of first-order reactions. Second-order rate constants were obtained as slopes of the lines k_1 vs. $[H^+]$. Mass spectra of the reaction products were recorded by use of the RP-HPLC/ESI–MS technique.

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