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The Oxidatively-Induced DNA Lesions 8,5'-Cyclo-2'-Deoxyadenosine and 8-Hydroxy-2'-Deoxyadenosine Are Strongly Resistant to Acid-Induced Hydrolysis of the Glycosidic Bond

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Abstract

The 8,5'-cyclopurine-2'-deoxynucleosides (cPu) are unique oxidatively-induced DNA lesions in that they are specifically repaired by NER. In the absence of NER, a possible mechanism for cPu removal is spontaneous glycosidic bond hydrolysis followed by enzymic processing. Such a mechanism could be significant if the glycosidic bond in cPu were substantially destabilized, as shown for other DNA lesions. Therefore, we investigated the stability of the glycosidic bond in a cPu, 8,5'(S)-cyclo-dA (S-cdA) against acid hydrolysis. For comparison, we also studied 8-OH-dA. We found that the glycosidic bond in S-cdA is ≈ 40 fold more resistant to glycosidic bond hydrolysis compared to dA. Interestingly, under the same conditions, the glycosidic bond in 8-OH-dA was even more stable than in S-cdA. These studies effectively rule out any mechanism for the removal of S-cdA or 8-OH-dA from DNA that requires spontaneous glycosidic bond hydrolysis, and further support the proposed role of cPu in the neurodegeneration observed in xeroderma pigmentosum patients who lack NER. Of broader significance, since NER does not function in non-transcribed DNA sequences of terminally differentiated cells, including neurons, cPu are expected to accumulate in such sequences even in individuals with normal NER, which could be important in the ageing process.

Introduction

The free radical theory of ageing (Harman, 1956) holds that ageing is the result of free radical reactions which are responsible for progressive deterioration of organisms over time. Reactive oxygen species (ROS) derived from the mitochondrion, have been a major focus of interest with regard to this theory in the ageing field (Harman, 2003). Most macromolecules in cells, including lipids, proteins, and RNA, are constantly turning over as a result of cycles of synthesis and degradation, which provides some protection against the accumulation of oxidation

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products. However, genomic DNA in terminally differentiated cells such as neurons does not turnover, meaning that the potential for accumulation of DNA damage in such cells is of particular significance.

DNA is protected by a variety of repair systems, including base excision repair (BER) and nucleotide excision repair (NER), which together remove the majority of DNA lesions resulting from ROS (Friedberg et al., 2005). In contrast to the BER pathway, the rate of NER varies in different regions of the genome. In dividing cells, the rate of NER of cyclobutane pyrimidine dimers (CPDs) is highest in transcribed DNA sequences (Bohr et al., 1985), and specifically on the transcribed strand of active genes (Mellon et al., 1987), whereas NER in non-transcribed sequences proceeds more slowly. This phenomenon is known as transcription-coupled NER (TC-NER) (Friedberg et al., 2005; Hanawalt, 1994). However, in terminally differentiated cells, such as neurons, NER occurs on both the transcribed strand and non-transcribed strand of active genes at the same rate, but CPDs in non-transcribed DNA are essentially not repaired (Nospikel and Hanawalt, 2000). This phenomenon was originally named differentiation-associated repair (DAR) (Nospikel and Hanawalt, 2002), but more recently, DAR has been redefined as domain-associated repair (Nospikel et al., 2006).

The majority of DNA lesions that result from the hydroxyl radical, including the well studied lesion 8-oxo-dG, are repaired by the BER pathway. However, one specific class of oxidatively induced DNA lesions, the 8,5'-cyclopurine-2'-deoxynucleosides are unique in that they are specifically repaired by NER (Brooks et al., 2000; Kuraoka et al., 2000). For this reason, most of the focus on these lesions has been on their possible role in neurodegeneration that occurs in some patients with the genetic disease xeroderma pigmentosum (XP) who lack the capacity to carry out NER (Brooks, 2006). However, in view of the evidence that DAR is operative in terminally differentiated cells such as neurons (described above), it follows that cyclopurines which form in non-transcribed regions of neuronal DNA would also not be repaired, even in normal individuals. This logic assumes that the efficiency of the human NER pathway for CPDs and 8,5'-cyclopurine-2'-deoxynucleosides is approximately equal, and experimental evidence indicates that this is in fact the case (Kuraoka et al., 2000).

In view of these considerations, it is important to determine whether cells have any other mechanism of removing these lesions aside from NER. Such mechanisms might include non-enzymic degradation, and/or "gratuitous" repair by enzymic pathways other than NER which evolved to deal with other DNA lesions. One of the most quantitatively important forms of DNA degradation is depurination, which results from the hydrolysis of the C1'-N9-glycosidic bond of the purine deoxynucleosides (Lindahl and Nyberg, 1972). Work from several laboratories has shown that some DNA adducts facilitate depurination by destabilizing the glycosidic bond (Baik et al., 2002; Chen et al., 2001; Lin et al., 2001; O'Brien and Ellenberger, 2003; Rogan et al., 1993; Shipova and Gates, 2005; Yermilov et al., 1995; Zahid et al., 2006). Therefore, if the presence of the 8,5'-bond in an 8,5'-cyclopurine-2'-deoxynucleoside causes a substantial increase in the rate of glycosidic bond hydrolysis, and if the resulting hydrolysis product could be enzymically processed further (e.g. by an AP endonuclease), then such a pathway could potentially represent a biologically significant mechanism of removing these lesions from the genome in the absence of NER. To address the first step of this hypothetical mechanism, we investigated the effect of the additional 8,5'-bond in (5'S)-8,5'-cyclo-2'-deoxyadenosine (S-cdA) (Figure 1) on the acid hydrolysis of the glycosidic bond. Since spontaneous depurination proceeds via acid-catalyzed hydrolysis of the glycosidic bond (O'Brien and Ellenberger, 2003), the stability of the glycosidic bond in S-cdA against acidic hydrolysis is directly relevant to the spontaneous process.

In addition to S-cdA, we also investigated the effect of another oxidatively-induced DNA modification, 8-hydroxy-2'-deoxyadenosine (8-OH-dA) (Figure 1), on glycosidic bond

hydrolysis. Similar to S-cdA, the C8 of the purine ring is chemically modified in 8-OH-dA. However, from an overall structural standpoint, 8-OH-dA is clearly more similar to 8-OH-dG, which is repaired by glycosylase initiated BER, than to the cyclopurine deoxynucleosides, although no glycosylase that removes this lesion efficiently has been identified to date. Therefore, we also investigated the stability of the glycosidic bond in 8-OH-dA to acid hydrolysis.

Materials and methods

Calf thymus DNA, 2'-deoxyadenosine (dA), and formic acid were from Sigma Chemical Co (St. Louis, MO). S-cdA and 8-OH-dA were from Berry & Associates (Dexter, MI). Aldehyde reactive probe (ARP) was from Dojindo (Kumamoto, Japan). Water from Omnisolv (EMD Chemical Inc., Gibbstown, NJ) and acetonitrile (Burdick & Jackson) were used as the solvents for liquid chromatography. All chemicals were used as received. Biomax5 5kD molecular weight cut-off filters (Millipore, Bedford, MA) were used for filtering off the enzymes after hydrolysis.

Formic acid hydrolysis experiments

A typical acid hydrolysis experiment for the kinetic analyses was conducted by treating 2 nmol of the nucleoside with 15 % formic acid in a 500 μ L volume. The samples were incubated at 37 °C for different length of time and hydrolysis was stopped by freezing the sample in dry ice. Prior to freezing, a known amount of cytosine was added to the samples as an internal standard for quantification purposes. The samples were later lyophilized using a freeze drier (Kinetics Thermal Systems, Stone Ridge, NY), dissolved in 100 μ L water, and 49 μ L were injected into an analytical HPLC. The amount of unhydrolysed nucleoside or product (base form or modified form) was calculated by measuring the area of each peak at 264 nm. dA, S-cdA and 8-OH-dA kinetics were followed in the same manner. The acid dependence experiments of dA and S-cdA were performed by treating 2 nmol of sample with varying concentrations of formic acid at 37 °C for 24 h.

Aldehyde reactive probe reaction

The lyophilized samples after acid hydrolysis dissolved in 100 mM tris buffer (pH 7.4) and treated with equal volume of 10 mM aldehyde reactive probe and incubated at 37 °C for 1 h. After the incubation, samples were directly injected in the analytical HPLC and the chromatogram was monitored at 264 nm.

High Performance Liquid Chromatography (HPLC)

HPLC analysis was carried out using a Varian analytical system (Prostar 210) with a UV detector (Prostar 310). The details of the instruments are given elsewhere (Theruvathu et al., 2005). A water-acetonitrile solvent system with a gradient elution program (A: 100% water, B: 100% acetonitrile; 0 min : 0% B, 20 min: 20% B, 24 min: 70% B, 34 min: 70 % B, 35 min: 0% B, 50 min: 0% B) starting with 100% water was used for the analysis. A Phenomenex prodigy 5U ODS(2) column (25cm $\times$ 0.46 cm, 5 μ m) used for separating the compounds.

Liquid Chromatography-Mass Spectrometry (LC/MS)

LC/MS analysis was done using 1100 B series system from Agilent technologies (Rockville, MD). The details of the set up are mentioned elsewhere (Jaruga et al., 2004). The samples for the LC/MS analysis were prepared by hydrolyzing S-cdA under the conditions indicated in the text. The samples were lyophilized, dissolved in water and analyzed by LC/MS.

Results

Depurination of dA can be readily quantified by monitoring the release of free adenine (Ade), which is clearly separable from dA by HPLC. However, studying the hydrolysis of the glycosidic bond in S-cdA presented a problem in that even if this bond was to undergo hydrolysis, the adenine base would still be attached to the sugar via the covalent 8,5'-bond. We considered the possibility that cleavage of the glycosidic bond in S-cdA might result in the formation of an altered form of the molecule, which could be separated from intact S-cdA by HPLC.

To address this possibility, we initially exposed pure S-cdA to 66% formic acid at 37 °C for 72 h, and analyzed the products by HPLC. Both DNA and dA are quantitatively depurinated under these conditions (data not shown). As shown in Figure 2, the incubation of S-cdA under these conditions resulted in a significant loss of the peak corresponding to intact S-cdA (retention time, 12.5 min), and in the appearance of an additional peak (retention time, 9.0 min) that eluted earlier than S-cdA (Figure 2b). The same additional peak was observed when S-cdA was exposed to 66% formic acid at 37 °C for 18 h (data not shown).

To verify that the early eluting peak resulting from acid treatment of S-cdA represents the hydrolysis product rather than some other modification, S-cdA was treated with acid as described above, and the products analyzed by LC/MS (Figure 3a). In addition to a small amount of S-cdA (which elutes at 17.3 min in this system), we observed two additional peaks: a major peak with a retention time of 12.5 min, and a minor peak, (y; retention time, 15 min). The major peak eluting at 12.5 min provided ions at m/z 290, 268, and 164 at a fragmentor voltage of 100 V. Since the protonated molecular ion (MH^+) of S-cdA is at m/z 250 (Figure 3c), the ion at m/z 268 corresponds to the MH^+ of the hydrolysis product of S-cdA representing addition of a H_2O molecule (18 Da) to S-cdA, whereas the ion at m/z 290 is the sodium adduct ion (MNa^+) of the hydrolysis product. Sodium ion adducts such as these are commonly observed in LC/MS. The ion at m/z 164 in both S-cdA and its hydrolysis product is from the same origin, namely the base moiety plus the CHOH portion of the sugar moiety. Although the yield of m/z 164 is very small compared with MH^+ in both S-cdA and its product, we note that the ratio of m/z 164 to m/z 250 is slightly higher in the spectrum of the hydrolysis product than in that of S-cdA at this fragmentor voltage (compare Figure 3b to Figure 3c).

When the fragmentor voltage was increased to 200 V, the pattern of the mass spectrum changed in both cases, indicating increased fragmentation (Figures 3d,e). In the mass spectrum of the hydrolysis product (Figure 3d), the MH^+ (m/z 268) is still one of the major ions along with m/z 290, 164 and 178, whereas in the mass spectrum of S-cdA, the m/z 164 ion is the most prominent ion and the MH^+ (m/z 250) is at a negligible level (Figure 3e). The ion at m/z 178 in Figure 3d may represent a fragment ion that is composed of the base moiety and the OH-C5'-H-C4'H group (plus an H) from the sugar moiety. Taken together, these results indicate that the peak eluting at 9.0 min under the analytical HPLC conditions (Figure 2) and at 12.5 min under the LC/MS conditions (Figure 3a), represents the major hydrolysis product of S-cdA. We subsequently refer to this product as hydrolyzed S-cdA (h-S-cdA). In addition, these results indicate that the 4'-5' bond in h-S-cdA is more stable against fragmentation in the mass spectrometer than that in S-cdA.

As noted above, in addition to h-S-cdA, we also observed another minor peak (y) eluting at 15.0 min under the LC/MS conditions (Figure 3a). The major ion in the mass spectrum of this compound was m/z 296 at the fragmentor voltage of 100 V, which may represent the MH^+ (data not shown). This peak most likely corresponds to a formate ester (see (LaFrancois et al., 1998) of the initially formed S-cdA hydrolysis product. However, since this was a minor peak

that was not detectable in our analytical HPLC conditions, we did not attempt to further characterize it.

h-S-cdA could conceivably have two structures that would be consistent with the mass spectra shown in Figure 3. The deoxyribose moiety could exist in either a ring closed form, 5-C-(6-amino-9H-purin-8-yl)-2-deoxypentofuranose [h-S-cdA(f)] or a ring-opened aldehyde form, 5-C-(6-amino-9H-purin-8-yl)-2-deoxypentose [h-S-cdA(p)] (see Figure 4). To address the possibility that at least some h-S-cdA exists in the ring-opened aldehyde form, h-S-cdA solution was neutralized with NaOH and incubated with aldehyde reactive probe (ARP) (Kubo et al., 1992), a compound which specifically reacts with DNA constituents containing aldehyde groups (Bennett and Kitner, 2006; Ide et al., 1993). As shown in Figure 4, incubation of h-S-cdA with ARP results in the disappearance of the h-S-cdA peak and simultaneous appearance of a new peak at a longer retention time of 17 min (Figure 4b). This later eluting peak is observed only in reaction mixtures containing acid-treated S-cdA and ARP but not with reactions of S-cdA and ARP (Fig. 4c and data not shown), indicating that it results from the reaction of ARP with an aldehyde group in acid-treated S-cdA. These results indicate that h-S-cdA contains an aldehyde group, consistent with the structure of the pentose form shown in Figure 4. It should be noted, however, that because the reaction of ARP with deoxynucleosides is irreversible, we cannot rule out the possibility that h-cdA exists in an equilibrium between h-S-cdA(f) and h-S-cdA(p) forms, though our results do indicate the such an equilibrium would favor the h-S-cdA(p).

Kinetics of acid hydrolysis of S-cdA

Having established a method to detect h-S-cdA, we were then able to investigate the rate of hydrolysis of the glycosidic bond in S-cdA as compared to dA. The fact that some amount of S-cdA remained intact after prolonged exposure to 66% formic acid at 37 °C, a condition in which normal purine nucleosides are quantitatively hydrolyzed, indicated that the presence of the 8,5'-bond substantially stabilized the glycosidic bond against acid hydrolysis. To estimate the extent of stabilization, based on the results of preliminary experiments, we chose 15% formic acid as the concentration in which to compare the depurination kinetics of S-cdA and dA. As shown in Figure 5A, both dA and S-cdA depurinated in 15% formic acid, albeit at very different rates. Specifically, the time for 50% depurination ($t_{1/2}$) under these conditions was 35 min for dA versus 17 h for S-cdA, corresponding to an approximately 30-fold difference in the rate of glycosidic bond hydrolysis.

A similar series of experiments were carried out by varying the acid concentration while maintaining using a constant time interval of 24 h. As shown in Figure 5B, the concentration of formic acid necessary to carry out 50% depurination of dA under these conditions was 0.2%, whereas that for S-cdA was 10%. This is a difference of approximately 50-fold, in reasonable agreement with the results from the kinetic studies described above. Together, these results indicate that the presence of the 8,5'-bond in S-cdA provides an approximately 40-fold stabilization of the glycosidic bond against acid hydrolysis when compared to dA.

The glycosidic bond in 8-OH-dA is stabilized against acid hydrolysis to even greater extent than in S-cdA

To compare the stability of the glycosidic bond in S-cdA against acid hydrolysis with the glycosidic bond in another oxidatively-induced purine involving the C8 of the purine ring, we treated 8-OH-dA with 15% formic acid for 24 h at 37 °C. Under these conditions, the glycosidic bond in S-cdA undergoes >50% hydrolysis (Figure 5). Surprisingly, we found that the 8-OH modification in 8-OH-dA rendered the glycosidic bond completely refractory to acid hydrolysis (compare Figures 6a and 6b) in contrast to what we had observed with S-cdA. These results

indicate that the 8-OH modification to dA results in much greater stabilization of the glycosidic bond against acid hydrolysis than the 8,5'-bond in S-cdA.

Previous studies have shown that exposure of 8-OH-dA to 60% formic acid at 140 °C resulted in the quantitative release of 8-OH-Ade (Dizdaroglu, 1998). In view of the unexpected results at 37 °C, we investigated whether we could release free Ade from 8-OH-dA under these conditions. As shown in Figure 6c, exposure of 8-OH-dA to 60% formic acid at 140 °C did release the free 8-OH-Ade, as expected from previous data.

Exposure of S-cdA to acid at 140 °C increases the resistance of the base moiety against fragmentation

Finally, in an attempt to cleave the 8,5'-bond in S-cdA, we tested the effect of exposing S-cdA to 60% formic acid at 140 °C for 30 min, and analyzed the products by LC/MS. Under these conditions, no peak corresponding to free adenine was detected. Instead, in addition to a small amount of S-cdA, we observed an additional peak that eluted after h-S-cdA and before S-cdA (Figure 7a). The mass spectrum of the compound represented by the early eluting peak was analyzed at two different fragmentor potentials as shown in Figures 7b and 7c. The main ion present under these conditions is m/z 250, which can be obtained by the release of a water molecule (18 Da) from the initially formed h-S-cdA. Notably, in contrast to what we have observed with both S-cdA or h-S-cdA, at a fragmentor potential of 100 V, no ion at m/z 164 could be detected in samples of S-cdA exposed to acid at 140 °C. At a fragmentor potential of 200 V, only a very small amount the m/z 164 ion was observed. These observations indicate that the treatment of S-cdA in acid at 140 °C actually *stabilizes* the adenine base against fragmentation in the mass spectrometer. We speculate that this stabilization is the result of a dehydration reaction at C5', resulting in the formation of a double bond between C4' and C5', as illustrated in Figure 7d.

Discussion

In the present work, we show that treating S-cdA with acid results in glycosidic bond hydrolysis, and that the resulting product, which we refer to as h-S-cdA, can be detected using HPLC and LC/MS. Using this approach, we analyzed the kinetics of the hydrolysis of the glycosidic bond in S-cdA by acid, and compared the kinetics of this process with those in dA. We found that the hydrolysis of the glycosidic bond in S-cdA was reduced by a factor of ~ 40 -fold compared to dA. This observation indicates that the presence of the 8,5'-bond in S-cdA strongly stabilizes the glycosidic bond against acid hydrolysis. When S-cdA was incubated in acid at 140 °C, no free Ade was released indicating that even under these extreme conditions, the C8-C5'-bond could not be cleaved. These observations further underscore the extraordinary chemical stability of 8,5'-cyclopurine-2'-deoxynucleosides.

In addition to S-cdA, we investigated the effect of the oxidation of the C8-position on glycosidic bond hydrolysis in a modified purine deoxynucleoside, namely 8-OH-dA. Surprisingly, we found that the glycosidic bond in 8-OH-dA was almost completely resistant to acid hydrolysis at 37 °C, significantly more so than we observed for S-cdA. However, when 8-OH-dA was exposed to acid hydrolysis at 140 °C, the lesion completely depurinated, as expected from previous results. Thus these two different chemical modifications of the C8 of the purine ring have significantly different effects on chemical stability against acid hydrolysis.

Chemical Considerations

The increased resistance to acid hydrolysis of 8-OH-dA compared to S-cdA can be attributed to the electron donating effect of the OH group at the C8. Because of the electron donating effect, the electron density can be moved from the C8 to the neighboring N9, resulting in the

C1'-N9-bond assuming a partial double bond character, which is most likely responsible for the additional stability of this bond. Also, consistent with our observations, Bialkowski et al. (1996) observed that 8-OH-dG was more resistant to glycosidic bond hydrolysis than dG under both neutral and acidic conditions. Other data in the literature are consistent with the hypothesis that for DNA adducts, depurination is either facilitated or inhibited depending upon whether the modification is electron-withdrawing or electron-donating, respectively (Laayoun et al., 1994). For example, the electron withdrawing nitro group facilitates the depurination of 8-nitroguanine (Yermilov et al., 1995) and 8-nitroxanthine (Chen et al., 2001).

In the case of S-cdA, there is no such electron-donating group near the N9 other than the common groups in S-cdA and 8-OH-dA. Instead, it is most likely the additional 6 membered cyclic structure that provides extra stability rather than an electron-donating effect. It is known that 6 membered cyclic structures possess more stability than open structures because of the presence of an additional ring in the molecule. Consistent with our observations, Lin et al. (1987) observed an ≈ 6 fold reduction in the rate of glycosidic bond cleavage in the ribonucleoside 8,5'-cA under acidic conditions. The more dramatic (≈ 40 -fold) effect that we observe may be due to the difference between a deoxyribonucleoside versus a ribonucleoside, and/or differences in hydrolysis conditions. To explain their results, Lin et al. (1987) proposed an anti-Bredt transition state, which would be applicable to our results as well.

Biological considerations

Since spontaneous depurination proceeds via acid-catalyzed hydrolysis of the glycosidic bond (23), the stabilizing effect of the 8,5'-bond in S-cdA on acidic hydrolysis of the glycosidic bond is directly relevant to the spontaneous process. Therefore, our observation that the glycosidic bond in S-cdA is ≈ 40 -fold more stable than in dA effectively excludes any mechanism for the removal of S-cdA from DNA that involves spontaneous glycosidic bond hydrolysis from consideration as a realistic possibility. Based on their structures and biological properties, a similar degree of stabilization can be expected for the other 8,5'-cyclopurine-2'-deoxynucleosides as well.

Two different laboratories were unable to detect any evidence for a glycosylase which can cleave the C1'-N9 glycosidic bond in cyclopurine deoxynucleosides, using nuclear extracts from rat brain (Brooks et al., 2000) or Hela cell extracts (Kuraoka et al., 2000). The method used by Kuraoka et al. (2000) could also have detected other types of repair events, including direct repair, as well. Given the ability of NER to repair these lesions, the selective pressure for the evolution of a back-up pathway involving either a specific glycosylase or a different enzyme that could cleave the strong C8-C5' bond is not readily apparent. Therefore, although the possibility that such an enzyme may exist in some cell type of some organisms cannot be formally ruled out, the totality of the evidence currently available supports the view that in mammals, NER is the only mechanism by which 8,5'-cyclopurine-2'-deoxynucleosides can be removed from genomic DNA in the absence of cell division.

The requirement for the NER to remove 8,5'-cyclopurine-2'-deoxynucleosides has two important implications. First, it further strengthens the case for cyclopurine deoxynucleosides as neurodegenerative DNA lesions in XP neurological disease (Brooks, 2006). Of broader relevance to the mechanisms of ageing, the absence of NER from non-transcribed regions of the genome in neurons due to DAR, coupled with our current results, means that cyclopurine deoxynucleosides would be expected to accumulate in non-transcribed sequences of neuronal DNA. This is significant, because S-cdA has been shown to completely block the binding of a transcription factor, the TATA binding protein, to its cognate recognition sequence, and a single S-cdA lesion in a TATA box has been shown to strongly reduce transcription in human cells (Marietta et al., 2002). Moreover, *in vitro* studies have shown that a single S-cdA lesion can prevent the binding of several other transcription factors to their recognition sequences as

well (P.J. Brooks, unpublished observations). Therefore, accumulation of 8,5'-cyclopurine-2'-deoxynucleosides in non-transcribed DNA sequences in neurons could reduce gene expression in neurons over time by blocking the binding of transcription factors to DNA. Such a mechanism seems well-suited to explain what happens in the brain during ageing, which is not a quantitative loss of neurons, but rather a decline in neuronal function (Long et al., 1999). To directly test this possibility, quantitative studies to examine the level of 8,5'-cyclopurine-2'-deoxynucleosides in neuronal DNA during ageing are clearly warranted. However, because neurons represent only about 10% of cells in the mammalian brain, with the remainder being glial cells, a method to purify neuronal DNA prior to analysis will be necessary.

There is clear evidence that 8-OH-dA lesions generated in cells by exposure to free radicals can be repaired *in vivo* (Jaruga and Dizdaroglu, 1996; Tuo et al., 2002), although the mechanistic basis for this repair process is unclear. Since an *in vitro* reconstituted NER system has been shown to act on DNA containing 8-OH-dG (Reardon et al., 1997), it is possible that 8-OH-dA could also be repaired by NER, though it seems unlikely to be a very avid substrate *in vivo*. Alternatively, a human 8-OH-dA glycosylase may remain to be discovered. While the *E. coli* Fpg protein has been shown to possess some minor activity for 8-OH-Ade removal from DNA (Boiteux et al., 1992), the homologous Ogg1 protein in eukaryotes does not exhibit any activity toward this lesion (Dherin et al., 1999). Determining whether 8-OH-dA is repaired by NER or BER or both is important from the point of view of the potential for this lesion to accumulate in non-transcribed DNA of terminally differentiated cells, and should be the subject of further investigation.

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Figure 1. Structures of 2'-deoxyadenosine (dA), (5'S)-8,5'-cyclo-2'-deoxyadenosine (S-cdA), and 8-hydroxy-2'-deoxyadenosine (8-OH-dA).

Figure 2. HPLC chromatogram (264 nm) of (a) S-cdA (2 nmole), (b) S-cdA (2 nmole) treated with formic acid (66%) for 72 h at 37 °C.

Figure 3.

LC/MS analysis of S-cdA, which was hydrolyzed using formic acid (60%) for 24 h at 37 °C. (a) Chromatogram recorded at 254 nm; (b) Mass spectrum of the h-S-cdA (retention time at 12.5 min) at a fragmentor voltage of 100 V; (c) Mass spectrum of the S-cdA at 100 V; (d) Mass spectrum of the h-S-cdA at 200 V. (e) Mass spectrum of the S-cdA at 200 V.

Figure 4.

Top: Reaction scheme for the formic acid hydrolysis of S-cdA at 37 °C, illustrating the possible products. Bottom: HPLC chromatogram of the reaction mixture of (a) S-cdA (2 nmol) treated with formic acid (20%) for 55 h at 37 °C; (b) S-cdA (2 nmol) treated with formic acid (66%) for 72 h and then lyophilized, neutralized and treated with 110 nmole of ARP at 37 °C for 1 h; (c) S-cdA (2 nmol) treated with ARP (110 nmol) and incubated at 37 °C for 1 h.

Figure 5.
 (a) Kinetics of dA (Δ) and S-cdA ($\bullet$) glycosidic bond cleavage in 15% formic acid at 37 °C. Inset: expanded view of dA kinetics. (b) Hydrolysis of S-cdA ($\bullet$) and formation of h-S-cdA ($\circ$) in different formic acid concentrations at 37 °C for 24 h.

Figure 6.

LC chromatogram of formic acid hydrolyzed 8-OH-dA at 254 nm : (a) 8-OH-dA without any treatment, (b) 8-OH-dA treated with 15% formic acid for 24 h at 37 °C, (c) 8-OH-dA treated with 60% formic acid for 30 min at 140 °C.

Figure 7.

Exposure of S-cdA to acid at 140 °C results in hydrolysis of the glycosidic bond as well as stabilization of the C4'-C5' bond. (a) Total-ion chromatogram of the reaction mixture of S-cdA after the hydrolysis by 60% formic acid at 140 °C for 30 min. (b, c) Mass spectra of the peak at 14.7 min at a fragmentor voltage of 100 V (b) or 200 V (c). (d) Proposed reaction scheme of formic acid hydrolysis of S-cdA at 140 °C.