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Efficacy and site specificity of hydrogen abstraction from DNA 2-deoxyribose by carbonate radicals

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Abstract

The carbonate radical anion $CO_3^{\bullet-}$ is a potent reactive oxygen species (ROS) produced *in vivo* through enzymatic one-electron oxidation of bicarbonate or, mostly, via the reaction of CO_2 with peroxynitrite. Due to the vitally essential role of the carbon dioxide/bicarbonate buffer system in regulation of physiological pH, $CO_3^{\bullet-}$ is arguably one of the most important ROS in biological systems. So far, the studies of reactions of $CO_3^{\bullet-}$ with DNA have been focused on the pathways initiated by oxidation of guanines in DNA. In this study, low-molecular products of attack of $CO_3^{\bullet-}$ on the sugar–phosphate backbone *in vitro* were analyzed by reversed phase HPLC. The selectivity of damage in double-stranded DNA (dsDNA) was found to follow the same pattern C4′ > C1′ > C5′ for both $CO_3^{\bullet-}$ and the hydroxyl radical, though the relative contribution of the C1′ damage induced by $CO_3^{\bullet-}$ is substantially higher. In single-stranded DNA (ssDNA) oxidation at C1′ by $CO_3^{\bullet-}$ prevails over all other sugar damages. An approximately 2000-fold preference for 8-oxoguanine (8oxoG) formation over sugar damage found in our study identifies $CO_3^{\bullet-}$ primarily as a one-electron oxidant with fairly low reactivity toward the sugar–phosphate backbone.

Keywords: carbonate radical, DNA, oxidative damage, 2-deoxyribose, hydrogen abstraction

Introduction

Until recently, the physiological role of ROS derived from the bicarbonate anion has been significantly underestimated despite the facts that the HCO_3^-/CO_2 pair constitutes the main physiological buffer and bicarbonate is present in high concentrations in living organisms (25 and 14 mM in serum and intracellular media, respectively). There is growing evidence, however, that the carbonate radical anion $CO_3^{\bullet-}$, also known as the "carbonate radical," a product of one-electron oxidation of HCO_3^-, plays a special role as an important ROS in normal cellular metabolism and a number of pathological conditions [1–3]. The best known biological role of $CO_3^{\bullet-}$ is associated with the modulation of peroxynitrite activity through the intermediate formation of the nitrosoperoxycarbonate anion, $ONOOCO_2^-$, which further undergoes homolysis to produce NO_2 and $CO_3^{\bullet-}$. The resulting $NO_2/CO_3^{\bullet-}$ couple is an efficient nitration system, in which $CO_3^{\bullet-}$ first abstracts hydrogen from amino acids in proteins, with a preference for tyrosine, and then addition of NO_2 occurs to yield nitro-substituted protein residues [1,4]. $CO_3^{\bullet-}$ may also be generated in cells as a product of bicarbonate or carbon dioxide oxidation at the active site of Zn, Cu SOD [5,6] or xanthine oxidase [7].

Although $CO_3^{\bullet-}$ is not as potent oxidant as the hydroxyl radical $HO^{\bullet}$ ($E^0 = 1.78$ V [8] vs. 2.3 V [9], respectively, at pH: 7), it nevertheless exceeds all other biologically relevant ROS in that respect. It has been demonstrated that $CO_3^{\bullet-}$ selectively oxidizes guanines in dsDNA by one-electron transfer with a rate of $\sim 5 \times 10^7\ M^{-1}\ s^{-1}$ at pH: 7.5 [10–12]. It may also act as a hydrogen abstractor although the rates of α-hydrogen abstraction from simple aliphatic alcohols have been reported to be 2–3 orders of magnitude slower [3,13]. A longer lifetime resulting from the lower reactivity toward hydrogen abstraction allows $CO_3^{\bullet-}$ to travel longer distances in biological media as compared with the extremely reactive hydroxyl radical [1]. This property potentially makes $CO_3^{\bullet-}$ no less harmful than the hydroxyl radical in cellular environment.

Until present all attention to $CO_3^{\bullet-}$ as a potential DNA damaging agent has been focused on its reactions with guanine. It has been demonstrated by the research group of Shafirovich [10–12] that $CO_3^{\bullet-}$ selectively reacts with guanine to produce the guanine radical G(-H)$^{\bullet}$ by a one-electron oxidation and is not reactive toward other DNA bases. Further transformations of guanine radicals produce unique end products of four-electron oxidation of guanine including 8oxoG as well as intrastrand crosslinks between guanine and thymine [12,14–16].

To the best of our knowledge, however, the reactivity of $CO_3^{\bullet-}$ toward the 2-deoxyribose moiety (dR) has never been discussed in the literature while the behavior of $CO_3^{\bullet-}$ in systems with similar reactivity toward hydrogen

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abstraction points at such a possibility. Extensive chain scission of glycosaminoglycans (long-chain linear polysaccharides) has been demonstrated by both hydroxyl radicals and $CO_3^{\bullet-}$ [17,18]. It has also been reported that $CO_3^{\bullet-}$ selectively attacks the weakest C1′–H bond in glucose [19], which is likely to be similar in reactivity to the same position in dR.

The aim of the present study is to evaluate the overall ability of $CO_3^{\bullet-}$ of inducing oxidative damage to the sugar–phosphate backbone via hydrogen abstraction from dR and the competitiveness of this process with oxidative damage to the nucleobases. Also we compare the preferences of $CO_3^{\bullet-}$ and $^{\bullet}OH$ in these two competing processes. $CO_3^{\bullet-}$ were generated photolytically from carbonatopentamminecobalt(III), $[Co(NH_3)_5CO_3]^+$, and the damage to dR was analyzed using the HPLC-based approach elaborated by our research team over the past few years (Figure 1). The contributions of the C1′ and C5′ pathways were evaluated from the yields of 5-methylenefuran-2(5H)-one (5MF) and furfural (Fur), respectively, which are the signature products of the C1′ and C5′ damages [20–22]. The contribution of the C4′ pathway was calculated from the combined yields of malondialdehyde (MDA) and N-oxycarbonylmethyl-5-methylene-Δ^3-pyrrolin-2-one (lactam or Lac), formed upon derivatization of the C4′-oxidized abasic site (C4′-OAS) with glycine [23]. Free base release (FBR) was employed as an alternative measure of dR damage since the formation of all of the above-mentioned products is necessarily accompanied by FBR when initiated by direct hydrogen abstraction from dR [24]. The production of 8oxoG was also monitored to evaluate the relative importance of guanine and dR oxidations in DNA damage inflicted by $CO_3^{\bullet-}$.

Methods

Reagents

Highly polymerized dsDNA from salmon testes (Sigma) was prepared and stored as a 10 mM (in nucleotides) stock solution in 10 mM phosphate buffer (pH: 6.9) at 4°C. Solutions of ssDNA were prepared from this stock by thermal denaturation (15 min at 90°C) with subsequent rapid cooling in ice. No appreciable strand recombination has earlier been observed upon storage of these solutions at 4°C for a period of at least 24 hours [22,23]. Carbonatopentamminecobalt(III) nitrate $[Co(NH_3)_5CO_3]NO_3$ hemihydrate was obtained from Sigma. It was converted into perchlorate by adding solid sodium perchlorate to its nearly saturated solution, and filtering off the precipitate thus formed. Concentrations of the complex in solutions were determined using its extinction coefficient of $\varepsilon = 70\ M^{-1}\ cm^{-1}$ at 506 nm [25]. Authentic

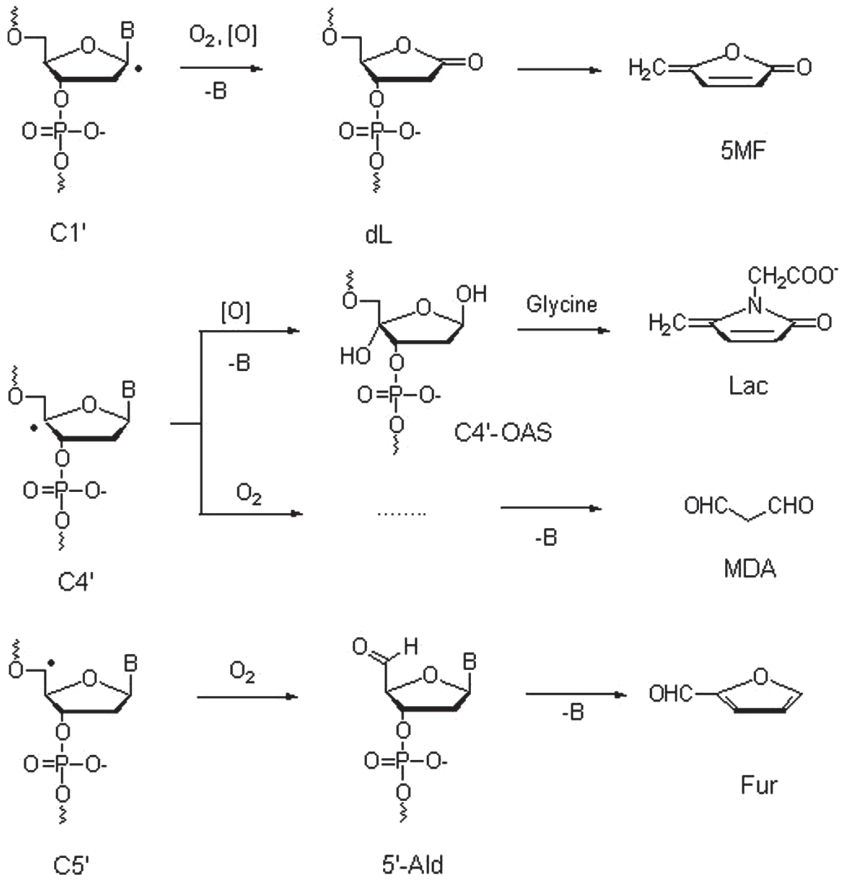

Figure 1. Characteristic end products of oxidative damage to dR analyzed in this study, and the mechanisms of their formation from free radical precursors. Abbreviations dL, C4′-OAS, and 5′-Ald stand for 2-deoxyribonolactone, C4′-oxidized abasic site, and oligonucleotide-5′-aldehyde, respectively.

8oxoG was prepared as described in the literature [26]. Ammonium acetate, protamine sulfate, spermine tetrahydrochloride, glycine, DNA bases, uracil, formic acid, thiobarbituric acid (TBA), and other reagents were of the highest available grade from Sigma-Aldrich or Fisher Scientific and were used as received.

Sample preparation and photolysis

Freshly prepared solutions (typically 800 μL) containing DNA (5 mM in nucleotides) and $[Co(NH_3)_5CO_3]^+$ (2 mM) in 10 mM phosphate buffer (pH: 6.9) were illuminated in 1.5-mL Pyrex vials for up to 8 min with vigorous stirring at room temperature under air. Vigorous stirring was necessary to keep the solutions air-saturated and to ensure homogeneity of the samples during photolysis. A 75 Xe lamp (PowerArc system from Optical Building Blocks Corporation) was employed as a UV source. The effective illumination wavelength was greater than 300 nm due to the absorption of short UV component of the source's emission in the glass. Intensity of the source in the photochemically effective spectral region 300 nm $< \lambda <$ 350 nm was estimated to be about 2×10^{18} photons $L^{-1}\ s^{-1}$ by ferrioxalate actinometry. After photolysis the samples were kept on ice until further treatment. Non-illuminated samples were used as the controls. All experiments were typically repeated several times for statistical analysis of experimental data.

Sample processing

A standard procedure of DNA hydrolysis with formic acid was employed in quantification of 8oxoG in the photolyzed samples [27]. Typically 200-μL aliquots were transferred into Wheaton glass ampoules and the DNA was precipitated with 20 ?L of 100 mM spermine hydrochloride. After centrifugation, the precipitate was washed twice with 10 mM phosphate buffer in the same ampules, the supernatants were discarded, and the precipitate was resuspended in 200 μL of 88% formic acid. The samples were degassed by a freeze–pump–thaw procedure, sealed, and heated for 90 min at 150°C. Formic acid was then removed in vacuum and the residue redissolved in 100 μL of 100 mM NaOH. After about 15 min of periodic agitation, the samples were diluted with 900 μL of 40 mM ammonium acetate and analyzed by reversed phase HPLC.

The products originating from oxidative damage to dR were analyzed as in our previous studies [22,23]. Briefly, 200-μL aliquots of illuminated samples were treated at 70°C with 10 mM spermine tetrahydrochloride (35 min) or 200 mM glycine (20 min) (all concentrations are final). The DNA was then precipitated with aqueous protamine sulfate (1.3 mg/ml in the final solutions), and the supernatants were analyzed by HPLC for FBR, 5MF, Lac, and Fur. MDA was analyzed using the TBA-reactive substances (TBARS) assay described elsewhere [28]. The colored MDA–TBA 1:2 adduct ($\lambda_{max} = 532$ nm, $\varepsilon_{max} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) formed in this reaction was quantified in the reaction mixture either by HPLC or photometrically, after extraction into 300 μL of n-butanol.

HPLC analysis

The samples were analyzed on a Prominence UFLC system (Shimadzu) equipped with a PDA detector, a degasser, a column oven, and an autosampler. All separations were performed on a Gemini C18, 250 X 4.6 mm column (Phenomenex) operated at 30°C and a 1 mL/min flow rate using 40 mM ammonium acetate as a running phase. The following linear gradients of acetonitrile were applied for elution of products: from 0 to 8.8% acetonitrile over 10 min for the analysis of 8oxoG; from 0 to 16% acetonitrile over 15 min for the analysis of FBR, Lac, 5MF, and Fur, and from 12 to 24% acetonitrile over 15 min for quantification of the MDA–TBA adduct. The concentrations of free bases, 5MF, Lac, and Fur were determined by comparing the corresponding peak area to extinction coefficient ratios with that of uracil (2 μM) employed as an internal standard [23]. The procedures for MDA and 8oxoG determinations were calibrated using the authentic compounds as references.

Results

The exposure of ssDNA or dsDNA in phosphate-buffered solutions to UV–Vis radiation ($\lambda > 300$ nm) in the presence of $[Co(NH_3)_5CO_3]^+$ produces easily detectable amounts of dR damage products while no effect is observed in the absence of the cobalt complex. Therefore, direct photolysis of the DNA can be excluded as a contributor to the observed effect. The representative chromatograms obtained by treatment of the photolyzed samples with glycine and spermine tetrahydrochloride are shown in Figure 2 (panels A and B, respectively). Both chromatograms clearly show the presence of Lac and 5MF along with all four free DNA bases. Uracil (U), which is also visible in these chromatograms, was added after photolysis as an internal standard for quantification purposes. The presence of Fur is more evident at the detection wavelength of 280 nm where its absorption maximum is located (data not shown). The yields of MDA were found to be relatively low both chromatographically and photometrically in these experiments ($\leq 10\%$ of the yield of Lac, data not shown).

Figure 3 shows accumulation of free bases (FBR) and the most abundant low-molecular-weight end products of dR oxidation as a function of exposure. In all cases, nearly linear time dependencies were obtained for at least the first 8 min of photolysis. The rates of formation of individual products (in μM/s) found from these plots were used to evaluate the relative product yields. The data thus obtained are shown in Table I as the percentage of the rate of FBR. The combined yield of 5MF, Fur, Lac, and MDA is designated in Table I as sugar damage products (SDPs). Experiments with ssDNA conducted in the same way revealed the formation of the same products but with a pronounced increase in the relative yield of 5MF release (Table I).

The chromatograms in Figure 4 show accumulation of 8oxoG as a function of exposure in the same samples. The

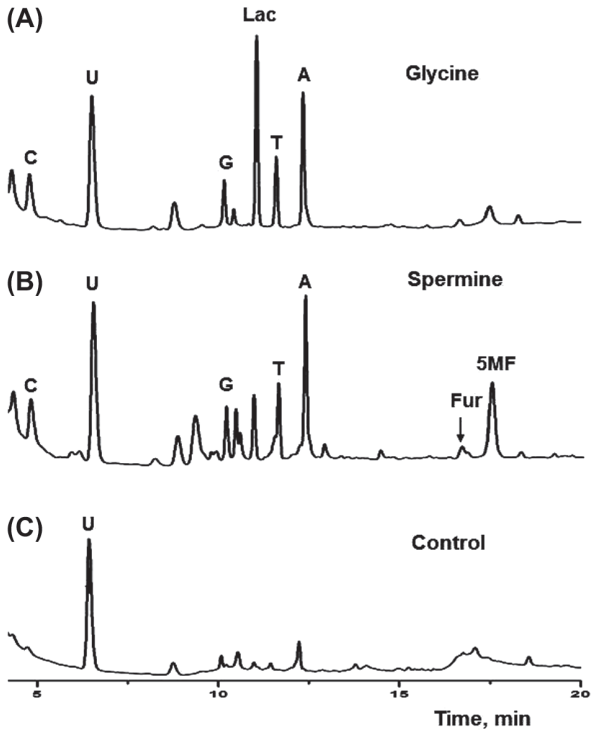

Figure 2. Representative chromatograms of dsDNA (5 mM) photolyzed for 8 min in the presence of carbonatopentamminecobalt(III) perchlorate (2 mM) in 10 mM phosphate buffer, pH 6.9. The samples were treated with 200 mM glycine (panel A) and 10 mM spermine tetrahydrochloride (panel B) at 70°C for 20 and 35 min, respectively. The control (panel C) shows the effect of spermine treatment on a sample not exposed to UV-light. Free bases (C, G, T, and A), Lac, 5MF, and Fur are labeled directly in the chromatograms. Uracil (U, 2 μM) was added after photolysis and employed as an internal standard.

detection wavelength of 305 nm was employed in these measurements to improve the 8oxoG-to-guanine peak intensity ratio. As follows from Figure 5, the concentration of 8oxoG quickly reaches a steady state corresponding to about 50 μM, or one 8oxoG per 50 base pairs. A model function $[8oxoG] = A(1\text{-}e^{-t/B})$, where t is the time of exposure while A and B are adjustable parameters, was employed to fit the data. This dependence describes accumulation of an unstable intermediate in two consecutive first-order

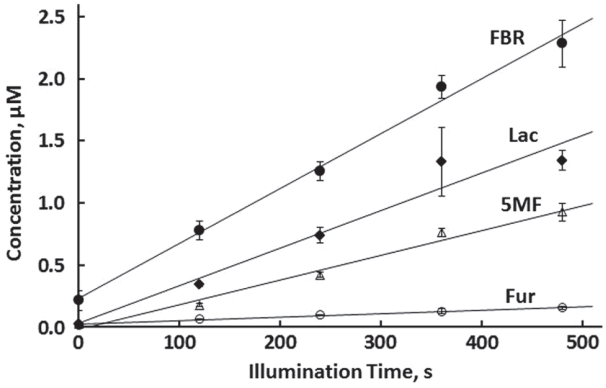

Figure 3. Accumulation of free bases (FBR) and characteristic low molecular weight products of dR damage (Lac, 5MF, and Fur) in the dsDNA/carbonatopentamminecobalt(III) perchlorate system as a function of UV-exposure.

Table I. Relative yields of oxidation products of DNA dR by hydroxyl radicals and by carbonate radicals represented as % of total FBR.

	Hydroxyl radicals from ref. [23]		Carbonate radicals	
Product	dsDNA	ssDNA	dsDNA	ssDNA
SDP	85	98	127	109
Lac	36	33	68	35
MDA	17	17	7	4
5MF	18	43	45	65
Fur	14	5	7	5

reactions with the rate-limiting first step that makes it appropriate in the present situation. Parameter A and the A/B ratio correspond to the steady-state concentration of the intermediate and the initial rate of its formation,

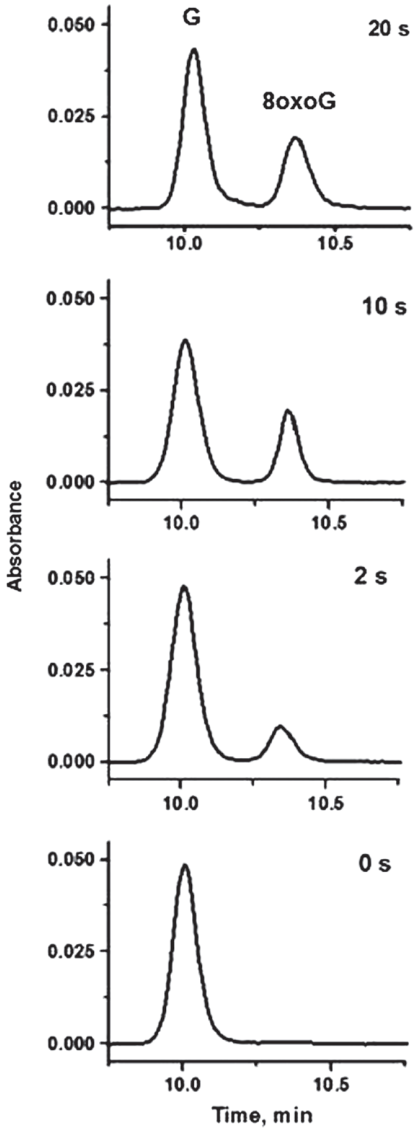

Figure 4. HPLC profiles showing the peaks of G and 8oxoG in the dsDNA/carbonatopentamminecobalt(III) perchlorate system for the first 20 s of exposure to UV light. The products were detected at 305 nm to improve the 8o × oG-to-guanine peak intensity ratio.

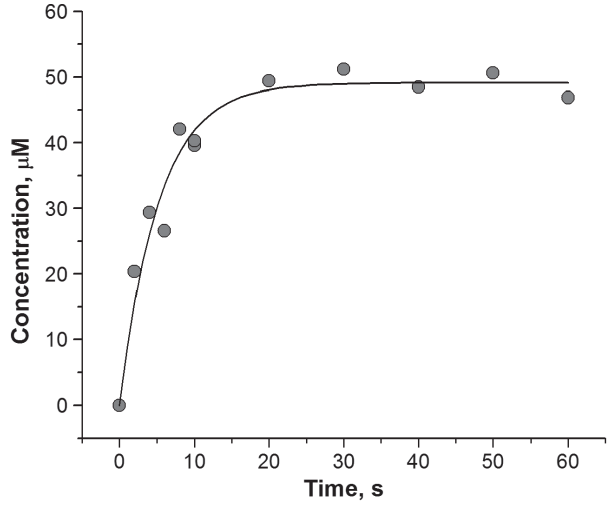

Figure 5. Accumulation of 8oxoG as a function of UV light exposure under the same experimental conditions as in Figure 5. Solid line is the best fit to the data obtained with a function $A(1\text{-}e^{-t/B})$ where t is the time of exposure, and A and B are adjustable parameters (see the Results section for more details).

respectively. The values of the parameters found from the fit were $A = 49 \pm 2$ μM and $B = 5.2 \pm 0.6$ s, which makes the initial rate of 8oxoG accumulation equal to about 9.3 μM/s. The formation of 8oxoG, therefore, is about 2000 times more efficient than FBR (rate of formation: 4.4 nM/s, Figure 3). This result identifies guanine oxidation as a predominant mechanism of DNA damage by $CO_3^{\bullet-}$.

Discussion

It has been demonstrated in a number of studies that photodissociation of $[Co(NH_3)_5CO_3]^+$ is a clean and convenient source of $CO_3^{\bullet-}$ for investigation of their reactions with DNA and its constituents [14,25]. The formation of $CO_3^{\bullet-}$ occurs with the quantum yield of about 0.06 upon photoexcitation of the complex into the charge-transfer band ($\lambda < 350$ nm) and is accompanied by ligand exchange [25].

$$[Co(NH_3)_5CO_3]^+ + h\nu \rightarrow Co^{2+} + 5NH_3 + CO_3^{\bullet-} \quad (1)$$

Since DNA absorbs negligibly at wavelengths greater than 300 nm, it does not interfere with the absorption of light by the complex in that spectral region. As expected, illumination of DNA alone did not form detectable amounts of damage products that allows for attributing the observed effect exclusively to photolysis of the complex. The linear product accumulation is consistent with the DNA damage being inflicted by primary photolysis products such as $CO_3^{\bullet-}$, and not through secondary photolysis of, for example, the product of ligand exchange.

The qualitative and quantitative aspects of the approach utilized in studies of dR damages in the present study have been discussed in detail in our previous publications. The release of 5MF and Lac from the corresponding precursors 2-deoxyribonolactone (dL) and C4′-OAS is quantitative or nearly quantitative [22,23]. Oxidation of the C4′-radical in oxygenated media also releases 3′-phosphoglycolates along with MDA; the latter being used as a quantitative measure of the contribution from this pathway [23]. Fur is released from oligonucleotide-5′-aldehydes (5′-Ald) whose formation is initiated by abstraction of the H5′-hydrogen with subsequent oxidation of the C5′-radical with molecular oxygen [22]. The chemical yield of Fur release from this precursor has not been measured, but the data of Chan et al. [29] suggest the 49% value as a lower limit estimate. We note that all the above-mentioned processes along with some not considered in this publication (in particular, those leading to 5-methylenefuran-3-one [30,31] and 1,4-dioxobutene [32]) result in unaltered base release. Therefore, the cumulative yield of all these products should not exceed that of FBR if all of them are formed through direct hydrogen abstraction from dR. Greater than FBR yields, however, can be observed if base-to-sugar damage valence transfer is involved (see below), and may serve as an indicator of the involvement of such a process.

Comparison of the products yields of dR damage by $CO_3^{\bullet-}$ and the hydroxyl radical (Table I) reveals several important differences between these two species. First, while both $CO_3^{\bullet-}$ and $^{\bullet}OH$ show the same general trend of hydrogen abstraction site preference in dsDNA, C4′ > C1′ > C5′, the relative yields of the products—and thus the contribution of each pathway–are quite different for these two species. The most pronounced difference is the greater relative role of the C1′-damage when the attack is initiated by $CO_3^{\bullet-}$. The ratio of the C4′ to C1′ oxidation decreases from ~ 2.9 for $^{\bullet}OH$ [23] to ~ 1.7 for $CO_3^{\bullet-}$ (present work), while the ratio of the C1′ to C5′ damage increases from ~ 1.3 to ~ 6.4, respectively. In ssDNA, abstraction of the H1′-hydrogen by $CO_3^{\bullet-}$ dominates over all other processes (65% of SDP), while the hydroxyl radical attack still results mostly in the H4′-abstraction, though with a lower relative yield than in dsDNA.

The increased preference of $CO_3^{\bullet-}$ attack at the C1′-position compared with the hydroxyl radical is generally consistent with $CO_3^{\bullet-}$ being more selective hydrogen abstractor. The C1′–H bond is the weakest C–H bond in the dR moiety [33,34], followed by the C4′–H and then C5′–H bonds. The selectivity of hydrogen abstraction by $CO_3^{\bullet-}$ from ssDNA follows that trend. The partial reversal of this order in favor of the H4′- abstraction in dsDNA is generally consistent with the lower accessibility of H1′ to free radical attack from the bulk. The insignificant role of H5′-abstraction by $CO_3^{\bullet-}$ for both dsDNA and ssDNA is also consistent with the C5′–H bond being the highest in energy in that row.

It should be noted that despite some preference toward inducing the C4′-damage, the relative efficiency of oxidation at the C1′-position still appears surprisingly high if direct abstraction of H1′ by the bulky and negatively charged $CO_3^{\bullet-}$ is considered the only source of it. This draws attention to the alternative base to sugar free valence transfer mechanism, suggested earlier by Xue and Greenberg [35] for an unexpectedly

efficient hydroxyl-radical-induced production of dL in dsDNA. According to this mechanism, the process is initiated by addition of the attacking radical to the C5-carbon of the pyrimidines with subsequent conversion of the carbon-based C6-radical into a peroxyl radical. Intramolecular hydrogen abstraction by the latter gives rise to the C1′-radical, which is further oxidized to form dL. While it is unknown whether $CO_3^{\bullet-}$ can add to the pyrimidine C = C bond, formation of very unstable addition complexes of $CO_3^{\bullet-}$ in reactions with unsaturated compounds has been proposed [3,36]. The possibility of the addition reaction of $CO_3^{\bullet-}$ with pyrimidines does not seem unreasonable since the closely related sulfate $SO_4^{\bullet-}$ and phosphate $HPO_4^{\bullet-}$ radical anions do enter this reaction [37]. In case of $CO_3^{\bullet-}$, a reproducible imbalance in the SDP and FBR yields in favor of the former observed in our study (Table I) indicates the possibility of this reaction. The imbalance results from the fact that the above-mentioned mechanism leads to the release of an oxidized base along with the formation of dL, not of an unaltered one as in the case of the direct H1′-abstraction. Further verification of this mechanism requires a closer look at the yields of altered bases in reaction of $CO_3^{\bullet-}$ with DNA, which is a subject of separate investigation.

It is noteworthy that an opposite imbalance in SDP and FBR is observed when the damage is initiated by $^{\bullet}OH$, which is known to abstract hydrogens directly from dR. In that case SDP is about 15% lower than FBR for dsDNA [23]. The difference can largely be attributed to dR damages not accounted for by the present analysis including the abstraction of H3′- and H2′-hydrogens, and is generally consistent with the nature of $^{\bullet}OH$ as an efficient and indiscriminate hydrogen abstractor.

Another striking difference between $^{\bullet}OH$ and $CO_3^{\bullet-}$ is the yields of MDA, the product of the alternative pathway of fragmentation of the C4′ radical. For $^{\bullet}OH$ the yield of MDA in dsDNA comprises almost 50% of the yield of Lac [23], while for $CO_3^{\bullet-}$ it is only ~ 10% (Table I). This difference cannot be explained by the selectivity in the hydrogen abstraction by these two reactive oxygen species since both MDA and Lac have the C4′-radical as a common precursor (Figure 1). It may originate, however, from oxidation of the C4′-radical by $[Co(NH_3)_5CO_3]^+$ in competition with its reaction with molecular oxygen. The C4′-carbocation thus formed is further hydrolyzed into the C4′-OAS, a precursor to Lac but not to MDA. The latter is believed to originate from a peroxyl radical intermediate since its formation takes place in oxygenated systems only.

Finally, our data show a nearly 2×10^3-fold preference for guanine oxidation into 8oxoG over hydrogen abstraction from dR in $CO_3^{\bullet-}$-induced DNA damage. This ratio is in agreement with the ratio of rate constants for the reaction of $CO_3^{\bullet-}$ with guanines in dsDNA ($(5 \pm 2) \times 10^7$ $M^{-1}\,s^{-1}$ [10–12]) and α-hydrogen abstraction from simple alcohols (10^4-10^5 $M^{-1}\,s^{-1}$) [38]. For radiation-produced hydroxyl radicals, which react with all DNA constituents at comparable rates, the balance is in favor of dR damage with the yield of 8oxoG (23 nmol J^{-1}) close to 50% of that of SDP (51 nmol J^{-1} [23]). Unlike FBR and SDP, the amount of 8oxoG in reaction of $CO_3^{\bullet-}$ with dsDNA quickly reaches a steady state at fairly low relative concentrations in DNA (about 50 base DNA pairs per one 8oxoG, Figure 5). The same phenomenon has previously been reported by Cai and Sevilla [39] in DNA oxidation by $Br_2^{\bullet-}$, whose oxidation potential ($E^o = 1.66$ V) is about the same as of the carbonate radical. It has been suggested that 8oxoG is further oxidized by mobile holes in the form of guanine radical cations $G^{\bullet+}$. The relative ease of 8oxoG oxidation ($E^o = 0.79$ V) compared with that of G ($E^o = 1.27$ V) is well known, and a number of products resulting from multiple guanine oxidation in DNA have been described (see ref. [37] for an extensive review).

In conclusion, the present study demonstrated for the first time the ability of $CO_3^{\bullet-}$ to inflict oxidative damage to dR at C1′-, C4′-, and C5′-positions. Although this process is far less efficient than oxidation of the guanines in DNA, its biochemical role may not be that negligible since, unlike the latter, it may lead to immediate and latent strand breaks.

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Declaration of interest

The authors report no conflict of interests. The authors alone are responsible for the contents and writing of the manuscript.

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