Reaction of Br_3^{2-} with 2-Deoxy-*D*-ribose. A Preferred Attack at C-1*

Barry J. Parsons**, Dietrich Schulte-Frohlinde, and Clemens von Sonntag

Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, Mülheim a. d. Ruhr

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In the photolysis of 5-bromouracil containing DNA Br atoms are expected intermediates. In order to evaluate the possible site of attack of the Br atom at the sugar moiety of DNA the reaction of 2-deoxy-*D*-ribose with the Br atom (complexed with two bromide ions) was investigated. Hydroxyl radicals generated by the radiolysis of N_2O saturated aqueous solutions were converted into Br_3^{2-} radicals by 1 M bromide ions. Br_3^{2-} reacts with 2-deoxy-*D*-ribose ($k = 3.7 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$, pulse radiolysis). The major product is 2-deoxy-*D*-erythro-pentonic acid ($G = 2.4$, γ -radiolysis). It is formed by hydrogen abstraction from C-1 and oxidation of this radical by other radicals. An alternative route via the radical at C-2 is negligible. It follows that Br_3^{2-} reacts preferentially at C-1 of 2-deoxy-*D*-ribose.

Introduction

In the photolysis of 5-bromouracil substituted DNA the C-Br bond is broken generating a uracyl radical and a Br atom [1, 2]. The uracyl radical appears to abstract a hydrogen atom from C-2' of a neighbouring nucleotide unit to give uracil and a radical at C-2 of the 2-deoxyribose unit [1]. These radicals are considered to be the precursors of DNA strand breaks [1, 3] most of which (> 80%) are of the "alkali-labile" type [4]. Recently a mechanism leading from the radical at C-2' to an alkali-labile strand break has been given [5].

The fate of the Br atom is still unknown. Especially one would like to know whether it could participate in the chain breaking process. In the present paper the reactions of the Br atom (complexed with two Br^- ions) with 2-deoxy-*D*-ribose have been studied. Although the complexation of the Br atom will reduce its reactivity and hence increase its specificity, major aspects of its reactions might nevertheless be gained from such a study.

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** Permanent address: The North Wales Institute of Higher Education, Kelsterton College, Connah's Quay, Clwyd CH 5 4BR.

Results and Discussion

Radiolysis of N_2O saturated aqueous solutions containing KBr (1 to 3 M) give rise to the intermediate Br_3^{2-} . The OH radicals which are generated according to reactions (1) and (2) rapidly react with Br^- to give Br atoms. Due to the high Br-concentration the Br atoms are complexed to form Br_3^{2-} [6].

Br_3^{2-} absorbs at 350 nm [6]. Using the pulse radiolysis technique the bimolecular decay of Br_3^{2-} (reaction (6)) and reactions with substrates (reaction (7)) can be monitored at this wavelength.

The rate of reaction of Br_3^{2-} with 2-deoxy-*D*-ribose has been studied at three 2-deoxy-*D*-ribose concentrations (10^{-1} , $2 \cdot 10^{-1}$ and $3 \cdot 10^{-1} \text{ M}$) and different KBr concentrations (1 to 3 M). The decay of the 350 absorption was of first order kinetics, the decay rate depending on the 2-deoxy-*D*-ribose but not on the KBr concentration. From these data a bimolecular rate constant of $(3.7 \pm 0.6) \cdot 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ was calculated for the reaction of

Br_3^{2-} with 2-deoxy-*D*-ribose. This rate constant is low compared to that of the OH radical with 2-deoxy-*D*-ribose ($k = 1.9 \cdot 10^9 \text{ l} \cdot \text{mol}^{-1} \text{ s}^{-1}$) [7] and it is to be expected that the selectivity of the Br_3^{2-} might be greater than that of the OH radical. The OH radical is known to abstract carbon-bound H atoms from carbohydrates approximately at random [8] producing a large number of products with similar G values. In the reaction of Br_3^{2-} with 2-deoxy-*D*-ribose only one major product was observed: 2-deoxy-*D*-erythro-pentonic acid ($G = 2.4$, γ -radiolysis). This value might be compared with a value of G (2-deoxy-*D*-erythro-pentonic acid) = 0.8 from OH attack (the value has been taken from ref. [9] and not from ref. [10] in order to have comparable irradiation and work-up conditions).

2-Deoxy-*D*-erythro-pentonic acid (3) might have two precursors, the radicals at C-1 (1) and C-2 (2). In the disproportionation reaction of the latter the enol form of the lactone (4) could be formed.

In order to check the possible contribution of this route 2-deoxyribosyl radicals were generated by OH attack and their reaction rate with $[\text{Fe}(\text{CN})_6]^{3-}$ measured by pulse radiolysis. There was a fast conversion of $[\text{Fe}(\text{CN})_6]^{3-}$ to $[\text{Fe}(\text{CN})_6]^{4-}$ with $G = 6$ ($k = 1.4 \cdot 10^9 \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$) and no detectable slow component. Studies on simple alcohols have shown that α -hydroxy alkyl radicals react at such a diffusion-controlled rate but β -hydroxy alkyl radicals two orders of magnitude slower [11]. Radical 2 is a β -hydroxy alkyl radical. The above experiments then show that only negligible amounts of radical 2 are formed by OH attack. Since the reactivity of Br_3^{2-} is much smaller than that of OH the attack of Br_3^{2-} at this position is expected

to be even less likely. The precursor of the 2-deoxy-*D*-erythro-pentonic acid (3) should therefore be radical 1.

Besides 3 three further minor products were observed, only one of which could be identified. It is 2,2-dideoxy-pentos-4-ulose.

Conclusions

It is concluded that Br_3^{2-} predominantly abstracts an H atom at C-1 of 2-deoxy-*D*-ribose. It is likely that the non complexed Br atom also shows selectivity. Br atoms are generated by the photolysis of 5-bromouracil substituted DNA. These Br atoms may add to the double bonds of the bases or may abstract hydrogen atoms from the sugar moiety giving rise to radicals at C-1'. Recently, it has been shown that H abstraction at this position induces alkali-labile DNA strand breaks [12].

Experimental

2-Deoxy-*D*-ribose and KBr were obtained from Merck and used without further purification. The water was triply distilled. N_2O (Hoechst) was freed from remaining traces of oxygen by passing through an Oxisorb column (Messer Griesheim). In pulse radiolysis $0.4 \mu\text{s}$ pulses of 20 to 1000 rad from 2.8 MeV Van de Graaff generator were applied [13]. Co-60- γ irradiations were done at a dose rate of $3 \cdot 10^{18} \text{ eV/g} \cdot \text{h}$. The doses used were up to $2 \cdot 10^{18} \text{ eV/g}$. In these experiments the 2-deoxy-*D*-ribose concentration was $5 \cdot 10^{-2} \text{ M}$. After irradiation the solutions were brought to dryness by rotary evaporation at 35°C and the carbohydrate products extracted with pyridine. 2-Deoxy-*D*-erythro-pentonic acid was determined as the trimethylsilyl ether of the acid as well as its γ - and δ -lactones. 2,3-Dideoxy-pentos-4-ulose was identified after reduction with NaBH_4 and trimethylsilylation by GC-MS [14]. The mass spectrum of this derivative was identical with that reported previously [14]. Separations were on a 40 m glass capillary column coated with OS 138 temperature programmed from 100 to 150°C by 1 K/min . For quantitative measurements erythritol was used as an internal standard. G(2-deoxy-*D*-erythro-pentonic acid) decreases with increasing dose. The value given in the paper was obtained by extrapolation to zero dose.

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