CHEMISTRY LETTERS, pp. 435-438, 1983. © The Chemical Society of Japan 1983

RADIATION CHEMISTRY OF DNA COMPONENTS. FORMATION OF THE 8,5'-CYCLO-2',5'-DIDEOXYGUANOSINE BY GAMMA IRRADIATION OF DEAERATED AQUEOUS SOLUTIONS OF 2'-DEOXYGUANOSINE AND ITS 5'-MONOPHOSPHATE ESTER

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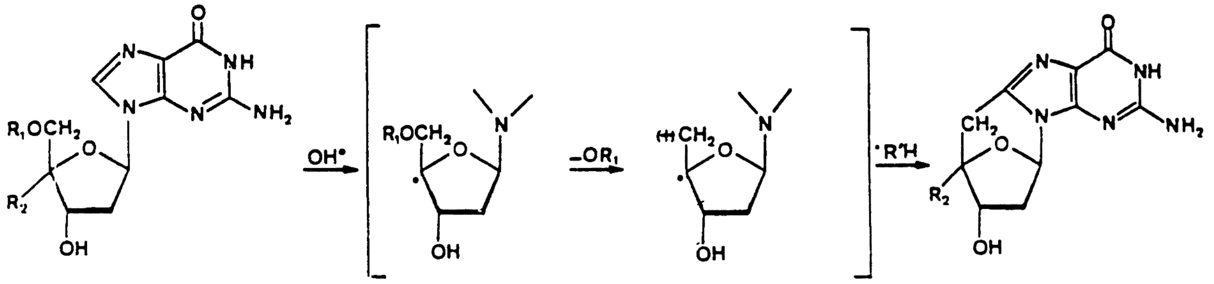
8,5'-cyclo-2',5'-dideoxyguanosine was shown to be produced by gamma irradiation of deaerated aqueous solutions of 2'-deoxyguanosine and 2'-deoxyguanosine-5'-monophosphate, subsequent to initial hydrogen abstraction at the osidic carbon C(4'). Structure assignment was made on the basis of UV, ^1H and ^{13}C NMR, mass spectrometry analyses, and confirmed by an independent synthesis.

Hydrogen abstraction from the deoxyribose ring of DNA chains is a common reaction initiated by hydroxyl radicals [1] and various radiomimetic agents [2]. Secondary reactions of the resulting sugar radicals lead in most cases to the rupture of the phosphodiester bond and/or to the cleavage of the N-glycosidic bond as shown by extensive studies involving DNA [3,4] and related model compounds, [1a,1b,5]. One major exception to these latter two processes [6] is the formation of 8,5'-cyclic derivative of 2'-deoxyadenosine [7] and 5'-AMP [8] through intramolecular aromatic substitution at C(8) by sugar radical produced at the C(5') position [9].

This paper reports a new radiation-induced reaction within the osidic moiety of 2'-deoxyguanosine (1a) and of its 5'-monophosphate ester (1b) which gives rise in both cases to 8,5'-cyclo-2',5'-dideoxyguanosine (3) in deoxygenated aqueous solutions [10]. The anhydronucleoside (3) was isolated and purified from a complex mixture of degradation products by reversed-phase high performance liquid chromatography (RPHPLC) [11]. Elemental analysis of the 3'-O-acetylated derivative of 3 deduced from exact mass measurement [12] (theoretical 291,0967 ; found 291,0972) was consistent with the empirical formula $C_{12}H_{13}N_5O_4$ which indicates the loss of the elements of H_2O from 1a. The base peak in the high resolution EI mass spectrum (m/e 251, 8-methylguanine $^+$) which results from the fragmentation of the deoxyribose ring at C(1) and C(4) is diagnostic [13] of covalent bond formation between imidazole C(8) and osidic C(5). As expected the 250 MHz ^1H NMR spectrum of 3 in D_2O [14] showed the lack of H(8) signal. The pronounced upfield shift of the H(5') and H(5") resonance signals and the marked increase in the magnitude of the geminal $J_{5',5''}$ coupling constant of 3 with respect to those of 1a indicate the absence of an hydroxyl substituent within the 5'-methylene group. Evidence for a rigid constrained structure was given by the low magnitude of the *trans* coupling constant $J_{1',2'}$ and $J_{3',4'}$ (< 1 Hz). Inspection of a Dreiding model shows that the corresponding H(1'),H(2') and H(3'),H(4') dihedral angles approach 90° with the furanoid ring in the unusual C(1')-*endo* O(4')-*exo* ($_0^1T$) conformation

[15] and that the orientation of the base is in the high *anti* range [16]. Confirmation of the structure of 3 was provided by its independent radiation-induced synthesis using 9-(5-bromo-2,5-dideoxy-β-D-*erythro*-pentofuranosyl)guanine as the starting nucleoside [17].

Hydroxyl radicals were shown to be the reactive water radiolysis species involved in the initial radical reaction leading to 3 [18]. Information on the nature of the resulting osidic radical was provided by experiments involving the radiation-induced formation of 3 from selectively prepared (4'-^{2}H)-2'-deoxyguanosine (2) [19]. The ^{1}H NMR spectrum of the corresponding cyclonucleoside (3) in D_2O showed a complete recovery of the H(4') signal due to the quantitative loss of the isotopic labelling. This result would strongly suggest that the radical arising from hydrogen abstraction at C(4') is the reactive intermediate.



1a R$_1$=H R$_2$=H

1b R$_1$=PO$_3$H R$_2$=H

2 R$_1$=H R$_2$=D

3 R$_2$=H

We consider it reasonable to propose a mechanism involving the transient formation of a radical cation [5b,20] consecutive to OH$^-$ or phosphate ester anion elimination from the initial nucleoside or nucleotide C(4') radical. Intramolecular cyclization with the rather nucleophilic carbon C(8) [21] and subsequent disproportionation reaction with other radicals ($^{\bullet}$RH) would lead to the anhydronucleoside (3) [22] (fig. 1). It is interesting to note that the radical at carbon C(4') derived from 1a is also involved in other competitive reactions giving rise to 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)guanine [23] and 9-(2-deoxy-β-L-*threo*-pentofuranosyl)guanine respectively through rearrangement of the osidic moiety [6] and epimerization of C(4') [24].

It has to be emphasized that the formation of 5,8'-cyclo-2',5'-dideoxyguanosine (3) by exposure of 5'-dGMP (1b) to γ-rays in aqueous deaerated solutions constitutes to our best knowledge the first example of a DNA base damage initiated by an osidic radical attack with concomitant release of the phosphoric ester group.

Acknowledgment. We thank H. Reutenauer (Centre Grenoblois de Résonance Magnétique) and J. Ulrich (Laboratoire de Spectrométrie de Masse, DCh/CEA) for their assistance in obtaining 250 MHz ^{1}H NMR and mass spectra respectively.

References

1) (a) J.F. Ward, Adv. Radiat. Biol. 5, 181 (1975). (b) C. von Sonntag and D. Schulte-Frohlinde, "Effects of Ionizing Radiations on DNA" ed. by J. Hüttermann W. Köhnlein, R. Teoule and A.J. Bertinchamps ; Springer-Verlag : Berlin, (1978), p 204 and references cited therein. (c) C. von Sonntag, Adv. Carbohydr. Chem. Biochem. 37, 7 (1980) and references cited therein.

2) (a) J.M.C. Gutteridge, FEBS Lett. 105, 278 (1979) (b) B.G. Que, K.M. Downey, and A.G. So, Biochem. 19, 5987 (1980) (c) L. Giloni, M. Takeshita, F. Johnson, C.Iden, and A.P. Grollman, J. Biol. Chem. 256, 8608 (1981).

3) (a) M. Dizdaroglu, C. von Sonntag, and D. Schulte-Frohlinde, J. Am. Chem. Soc. 97, 2277 (1975) (b) M. Isildar, M.N. Schuchmann, D. Schulte-Frohlinde, and C. von Sonntag, Int. J. Radiat. Biol. 40, 347 (1981).

4) (a) J.E. Strong and S.T. Crooke, Cancer Res. 38, 3322 (1978). (b) E.A. Sausville, R.W. Stein, J. Peisach, and S.B. Horwitz, Biochemistry , 17, 2746 (1978). (c) L.F. Povirk, Biochemistry 18, 3989 (1979).

5) (a) J.F. Ward and I. Kuo, Int. J. Radiat. Biol. 23, 543 (1973). (b) M. Dizdaroglu, K. Neuwald, and C. von Sonntag, Z. Naturforsch. 31b, 227 (1976).

6) Radiation-induced isomerization of the osidic ring of 2'-deoxyadenosine gives rise to a mixture of the four α and β furanoid and pyranoid anomers (N. Mariaggi, R. Teoule, J. Cadet, H. Dickie, and E. Hughes, Radiation Res. 79, 431(1979)) through acyclic sugar intermediate (J. Cadet and R. Teoule, J. Am. Chem. Soc. 96, 6517(1974).

7) (a) N. Mariaggi, J. Cadet, and Teoule, Tetrahedron 32, 2385(1976). (b) R. Teoule and J. Cadet, "Effects of Ionizing Radiations on DNA", ed. by J. Hüttermann, W. Köhnlein, R. Teoule, and A.J. Bertinchamps, Springer-Verlag : Berlin, (1978) p 171.

8) (a) K. Keck, Z.Narturforsch. 23b, 1034 (1968). (b) J.A. Raleigh, W. Kremers, and R. Whitehouse, Radiat. Res. 65, 414 (1976).

9) This cyclization process does not occur for dGuo (1a). One of the major final products derived from sugar radical at C(5') was assigned as 9-(2-deoxy-β-D-erythro-pento-1,5-dialdo-1,4-furanosyl) guanine. Thymidine-5'-aldehyde was shown to be produced at DNA strand breaks by the neocarzinostatin antibiotic (L.S. Kappen, I.H. Goldberg, and J.M. Liesch, Proc. Natl. Acad. Sci. USA, 79, 744 (1982).

10) 1 mM 2'-deoxyguanosine (1a) unbuffered aqueous solutions (pH 6.0) were irradiated in a ^{60}Co source to a dose of 4500 Grays at a dose rate of 150 Grays/min as measured by Fricke dosimetry (G(Fe^{+3}) = 15.5). Aqueous solutions of 1a or 1b were freed from oxygen by purging with N_2 for 30 min or by several freeze-pump cycles prior to gamma irradiation.

11) Reversed phase HPLC was conducted on a capped octadecylsilyl silica gel ODS-3 Whatman (25 x 0.46 cm I.D., mean particle size 10μm) column using water as the eluent ; capacity factors (k') for 1a and 3 are respectively 23.5 and 26.1.

12) Electron impact MS : m/e (rel intensity %) 291 (30 %, M^+), 231 (100 %, $M^+ - CH_3COOH$), 202 (42 %, M^+ $-CH_3COOH,HCO$), 185 (6%), 165 (71%), 123 (4%), 81(3%).

13) A. Matsuda, K. Muneyama, T. Nishida, T. Sato, and T. Ueda. Nucleic Acid Research 3, 3349 (1976).

14) 250 MHz ^{1}H NMR (D_2O, TSP) 6.36 (dd, 1H, $J_{1',2'}$ = 0.6 Hz, $J_{1',2''}$ = 5.2 Hz, $C_{1'}$-H), 2.71 (ddd, 1H, $J_{2',2''}$ = -14.0 Hz, $J_{2',3'}$ = 7.2 Hz, $C_{2'}$-H), 2.23 (ddd, 1H, $J_{2'',3'}$ = 3.4 Hz, $C_{2''}$-H), 4.54 (ddd, 1H, $J_{3',4'}$ = 0.6 Hz, $C_{3'}$-H), 4.84 (ddd, 1H, $J_{4',5'}$ = 6.5 Hz, $J_{4',5''}$ = 0.7 Hz, $C_{4'}$-H), 3.41 (dd, 1H, $J_{5',5''}$ = -17.9 Hz, $C_{5'}$-H) ; 2.99 (dd, 1H, $C_{5''}$-H) ; 62.8 MHz ^{13}C NMR (DMSO-d_6, TMS) 83.4 (d, $C_{1'}$), 82.4 (d, $C_{4'}$), 73.2 (d, $C_{3'}$), 46.1 (t, $C_{2'}$), 29.9 (t, $C_{5'}$).

15) T.P. Haromy, J. Raleigh, and M. Sundaralingam, Biochemistry, 19, 1718 (1980).

16) A. Matsuda, M. Tezuka, K. Niizuma, E. Sugiyama and T.Ueda, Tetrahedron, 34, 2633 (1978).

17) The 5'-halogenonucleoside was prepared according to an adaptation of a reported procedure (L.M. Beacham, J. Org. Chem., 44, 3100 (1979)) ; Radical reduction of 5'-iodo derivative of adenosine (K.N.V.Duong, A.Gaudemer, M.D.Johnson, R.Quillivic, and J.Zylber, Tetrahedron Lett., 2997 (1975)), and UV photolysis of 5'-phenylthiopurine nucleoside derivatives[13] represent alternative procedures to prepare the corresponding 5'-deoxy-5',8-cyclonucleosides.

18) The presence of 0.5 M methanol in the irradiated solution of 1a prevented the formation of 3.

19) The selectively 4'-deuteriated nucleoside (isotopic label up to 95 %) was prepared (yield 60 %) by warming 2'-deoxyguanosine-5'-aldehyde (K.E. Pfitzner and J.G. Moffatt, J. Am. Chem. Soc., 85, 3027 (1963)) in D_2O-pyridine (1:1) for 1 hr at 60°C (J. Cadet and M. Berger, in preparation), and subsequent $NaBH_4$ reduction.

20) L. Stelter, C. von Sonntag, and Schulte-Frohlinde, Z. Naturforsch., 30b, 656 (1975).

21) R. Lavery and Pullman, Theoret. Chim. Acta (Berl.), 53, 175 (1979). Radical cation has been proposed as the key intermediate in the binding reaction of the carcinogen N-acetoxy-2-acetamidofluorene to purine derivatives (J.D. Scribner and N.K. Naimy, Cancer Res., 33, 1159 (1973).

22) 1-(2,5-dideoxy-β-D-erythro-pentofuranos-5yl) uracil radical was recently characterized by ESR as a spin adduct, it was thought to result from radiation induced 4'-nucleosidic radical derived from 5-BrdUrd (M. Kuwabara, Y. Lion, and P. Riesz, Int. J. Radiat. Biol., 39, 491 (1981)). Related methyl radical at C(5) was shown to be produced by UV photolysis of hydrogen peroxide solution of ribose-5-monophosphate. However the corresponding radical was not observed with pyrimidine nucleosides and nucleotides (M. Kuwabara, Zhang Zhi-Xi, and G. Yoshii, Int. J. Radiat. Biol., 41, 241 (1982).

23) The α anomer of 1a could also result from anomerization of the $C_{1'}$ radical and subsequent disproportionation reaction.

24) The radiation chemical yield is expressed in terms of G value (molecule formed per 100 eV of energy absorbed) : $G_{(3)} = 0.09$ (0.8%) ; $G_{(9\text{-}(2\text{-deoxy-}\alpha\text{-D-erythro-pentofuranosyl})\text{guanine})} = 0.03$ (0.27%) ; $G_{(9\text{-}(2\text{-deoxy-}\beta\text{-L-threo-pentofuranosyl})\text{guanine})} = 0.05$ (0.44%) ; $G_{(2'\text{-deoxyguanosine-}5'\text{-aldehyde})} = 0.07$ (0.62%) ; $G_{(1a)} = 0.60$ (5.3%).

(Received December 24, 1982)