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Identification of Sugar Degradation Intermediates in a Metalloporphyrin-mediated DNA Cleavage Resulting from Hydroxylation at C-5'

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Hydroxylation of poly(dA)·poly(dT) at the 5' position of the deoxyribose units by the chemical nuclease Mn-TMPyP–KHSO₅ [Mn-TMPyP = manganese(III) complex of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin] followed by an additional thermal step provides 5'-aldehyde-3',4'-unsaturated nucleosides which can be reduced by sodium borohydride and then hydrogenated with palladium on charcoal giving 2',3'-dideoxyribonucleosides having the natural β or the non-natural α configuration at 4'.

Activation of C–H bonds of DNA sugars leading to DNA cleavage is a key step shared by several antitumour agents or chemical nucleases. Abstraction of a hydrogen atom is performed at C-1' by bis(*o*-phenanthroline)copper¹ or metal-loporphyrins,² at C-4' by activated bleomycin,³ at both C-1', C-4' and C-5' by neocarzinostatin⁴ and calicheamicin derivatives⁵, and more recently at C-5' by a manganese porphyrin.⁶ In this latter case, furfural (**FUR**) has been identified as the sugar degradation product generated after a thermal step required to trigger the β -elimination steps as described in Scheme 1. Here we report the chemical identification of the precursors of furfural after the hydroxylation of one of the C–H bonds at 5'-position of DNA sugars. Activated Mn-

TMPyP (*i.e.* oxidized by potassium monopersulfate, a watersoluble oxygen atom donor) is capable of abstracting a hydrogen atom at C-1' on CG rich sequences or at C-5' on AT rich sequences.⁶ Since poly(dA)·poly(dT) is mainly attacked at C-5' (83% compared to 17% at C-1'), this duplex was used in the present study to identify the furfural precursors. Hydroxylation at C-5' liberates a 3'-phosphate monoester and a 5'-aldehyde end (noted as 5'-ald end in Scheme 1).† The elimination of the phosphate from the 3' position of the

 $[\]dagger$ This step corresponds to a single-strand break on DNA without direct liberation of free base, whereas an hydroxylation at C-1' is always signed by a free base release.



Scheme 1 Proposed mechanism of DNA cleavage by hydroxylation at a C-H bond at 5' of deoxyriboses. Involvement of 5'-aldehyde-3',4'-dehydro-2',3'-dideoxynucleoside 1A and 1T as key intermediates in the formation of furfural, by reduction to the corresponding 2',3'-dideoxyribonucleosides 3 and 4.



Fig. 1 HPLC chromatograms of poly(dA)·poly(dT) after cleavage by Mn-TMPyP-KHSO₅. (a) After 3 min at room temp.; (b) after HEPES treatment and a heating step of 5 min at 90 °C and (c) after further treatment with NaBH₄ (...> indicate the t_R of reference compounds).

5'-aldehyde nucleoside to give 1 requires a thermal activation step.

Analysis of cleavage reaction‡ was performed on poly- $(dA) \cdot poly(dT)$ (700 µmol dm⁻³ nucl.) in 100 mmol dm⁻³ NaCl, 34 mmol dm⁻³ phosphate pH 8 buffer after cleavage by Mn-TMPyP (35 μ mol dm⁻³) in the presence of KHSO₅ (2 mmol dm-3; potassium monopersulfate is the triple salt 2KHSO₅, KHSO₄, K₂SO₄ known by the trade names Oxone, Curox or Caroat and is available from Aldrich or Alfa-Ventron). After 3 min of incubation with the chemical nuclease, the oxidant excess is destroyed by 100 mmol dm⁻³ HEPES [N'-(2-hydroxyethyl)piperazine-N-ethanesulfonic acid] pH 8 buffer. At this stage only small amounts of the free bases adenine A and thymine T [relative retention time (RRT) = 0.24 and 0.16], mainly produced by hydroxylation at C-1' were detected [Fig. 1(a)]. After a heating step at 90 °C for 5 min [Fig. 1(b)] an enhancement of free bases can be noted on HPLC chromatograms as well as the presence of four new peaks: FUR and 5-methylenefuran-2-one (5-MF), the markers for hydroxylation at C-5' and C-1' respectively, and two extra peaks noted 1T and 1A (RRT = 0.39 and 0.64). These two peaks are precursors of free bases and furfural, since they progressively disappear after a longer incubation period at 90 °C to the advantage of free T (1T), free A (1A) and furfural. These data suggest that the rate of the formation of furfural from the precursor 1 is slower than the β -elimination of the phosphate from C-3' ($k_2 < k_1$ in Scheme 1).

At this stage, the treatment of intermediates 1A and 1T for 30 min with NaBH₄ (final concentration 0.1 mol dm⁻³, room temp.; eventually the NaBH₄ was destroyed with acetone in excess) was supposed selectively to reduce the aldehyde at 5' to the alcohol without affecting the double bond, generating then the two new nucleoside derivatives 2T and 2A [Fig. 1(c), FUR and 5-MF which are reduced by sodium borohydride are not detected at 254 nm]. Their corresponding two HPLC peaks (RRT = 0.56 and 0.93) did not coelute with authentic samples of the 2',3'-dideoxynucleosides 3T/4T (these two isomers could not be separated in the used chromatographic conditions, RRT = 0.52, 3A (RRT = 1.00) and 4A (RRT = 1.00) (0.85). In order to confirm only the reduction of the aldehyde function of precursors 1 by NaBH₄, the intermediates 2T and 2A were collected after separation by HPLC and hydrogenated on palladium over charcoal within 10 min at room temp. The reaction was followed on HPLC equipped with a diode array detector allowing comparison of UV spectra of the different peaks. Interestingly, the hydrogenation reaction of 2A gave two isomeric products corresponding respectively (same RRTs and UV spectra, λ_{max} 260 nm) to authentic 3A and 4A isomers whereas the hydrogenation reaction of 2T

§ Compounds **3A** and **3T** (Sigma). Synthesis of 9-(α-L-5-hydroxymethyl-2-tetrahydrofuranyl)adenine **4A** was performed from 9-(5-*Otert*-butyldimethylsilyl-2-deoxy-α-L-*erythro*-pentofuranosyl)adenine according to general procedures previously described (C. Génu-Dellac, G. Gosselin, F. Puech, J. C. Henry, A. M. Aubertin, G. Obert, A. Kirn and J. L. Imbach, *Nucleosides Nucleotides*, 1991, **10**, 1345). Selected data for **4A**: ¹H NMR ([²H₆]DMSO) 1.83 (m, 1H, 3'-H), 2.23 (m, 1H, 3"-H), 2.45 (m, 2H, 2'-H and 2"-H partially obscured by [²H₅]DMSO), 3.43 (m, 2H, 5'-H and 5"-H), 4.38 (m, 1H, 4'-H), 4.78 (br s, 1H, 5'-OH), 6.27 (dd, 1H, $J_{1',2'}$ 4.9, $J_{1',2''}$ 6.2 Hz, 1'-H), 8.13 and 8.26 (2s, 1H each, 2-H and 8-H); MS (FAB > 0) 236 (M + H)⁺, 136 (BH₂)⁺; UV (H₂O) λ_{max} 260 nm (14800), λ_{min} 225 nm (2000). Satisfactory elemental analyses were obtained. For preparation of **4T** see the following reference: C. Genu-Dellac, G. Gosselin, A. M. Aubertin, G. Obert, A. Kirn and J. L. Imbach, *Antiviral Chem. Chemoth.*, 1991, **2**, 83.

[‡] HPLC conditions: 5μ nucleosil C₁₈ column eluted with 0.1 mol dm⁻³ triethylamine acetate pH 6.5 buffer, 5% acetonitrile; peaks were detected at 254 nm and characterized by their relative retention times (RRT = $t_{\rm R}$ of compound/ $t_{\rm R}$ of 3A): T, 0.16; A, 0.24; FUR, 0.31; 5-MF, 0.34; 1T, 0.39; 3T/4T, 0.52; 2T, 0.56; 1A, 0.64; 4A, 0.85; 2A, 0.93; 3A, 1.00 (see Fig. 1).

[¶] Compounds 2T and 2A were separated and collected from HPLC analysis of a tenfold scale experiment. The collected fractions were evaporated to dryness and dissolved in methanol (2T) or ethanol (2A). Hydrogen reduction on Pd/C of 2T and 2A (around 10 nmol of each compound) was complete within 10 min at room temp. Adsorption of the hydrogenation products on the solid catalyst lowered the yields of reduction to 80% for 2T and 40% for 2A. Preliminary experiments with authentic 2',3'-dideoxynucleosides confirmed that these compounds can be directly analysed on GC-MS without derivatization.

gave only one peak similar (same retention time and UV spectrum, λ_{max} 265 nm) to the peak corresponding to the unseparated standard samples of 2',3'-dideoxynucleoside 3T and 4T. For this reason the stereoselectivity of the hydrogenation of 2T cannot be ascertained from this reaction as in the case of 2A (see below). However, the GC-MS data of the hydrogenation product of 2T were identical to those of the authentic sample of 3T: the parent peak was observed at 226 m/z with the saturated dideoxysugar fragment at 101 (molecular mass of 3'-deoxythymine = 226). These observations support a 2',3'-dideoxynucleoside structure for the hydrogenation product of 2T but do not show unambiguously its exact nature (3T, 4T or a mixture). In the case of hydrogenation of 2A, 3A was always the major reaction product (3A: 4A ratio =60:40) indicating that the heterogeneous reaction mainly provides the isomer with the classical β configuration at 4'.

The proposed C-5' hydroxylation route for DNA cleavage by the chemical nuclease Mn-TMPyP-KHSO₅ is now supported by the chemical identification of derivatives of 5'-aldehyde-3',4'-dehydro-2',3'-dideoxynucleosides 1 which are the key intermediates in the release of furfural. This reaction pathway has been previously proposed by Goldberg and coworkers in a study on neocarzinostatin-mediated DNA breaks using tritiated nucleic acids. The 5'-aldehyde-3',4'unsaturated nucleoside 1 was initially proposed as a possible intermediate generated after a nuclease/alkali work-up of the drug-treated DNA.^{4a} As the first consequence of a C-5' hydroxylation, DNA cleavage is induced at ambient temperature with the formation of a 5'-CHO group that can be reduced to the corresponding alcohol derivative by NaB-H₄.^{4a,b} This reduction prevents the release of furfural. In these conditions, it must be noted that the oxidative action of Mn-TMPyP-KHSO₅, directly followed by a NaBH₄ reduction (without heating step), is generating two DNA fragments ending respectively with 5'-OH and 3'-phosphate termini, which is reminiscent of phosphodiester hydrolysis by some natural endonucleases, like DNAse II.

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 $[\]parallel$ GC-MS data were obtained with 2 µg of **3T** diluted in 3–5 µl of methanol before injection. Analyses were carried out with a Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5970 mass spectrometer working at 70 eV. Separations were obtained on a capillary column (12.5 m × 0.20 mm i.d. coated with crosslinked methylsilicone) with a 150 to 300 °C gradient (20 °C min⁻¹). Injector temperature was 250 °C.