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MILD ACID HYDROLYSIS OF 2-PYRIMIDINONE-CONTAINING DNA FRAGMENTS GENERATES APURINIC/APYRIMIDINIC SITES

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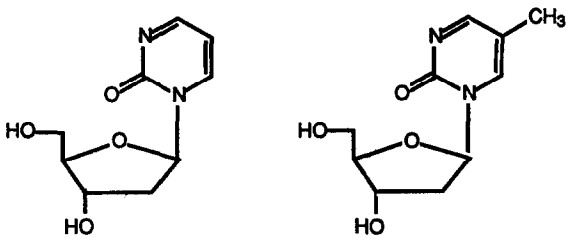
ABSTRACT Facile glycosidic bond cleavage of 2-pyrimidinone-2'-deoxynucleosides occurs under mildly acidic conditions (pH 3.0) and ambient temperature. When a 2-pyrimidinone nucleoside residue is present in a DNA fragment, hydrolysis results in an apurinic/apyrimidinic (abasic) site. The incorporation of 2-pyrimidinones into chemically synthesized DNA provides a route for the chemical generation of an abasic site at a preselected position in the sequence.

Cleavage of the N-glycosyl bond between a base and its 2'-deoxyribose moiety in DNA generates an apurinic/apyrimidinic (abasic) site. The phosphate-deoxyribose chain remains intact but the sequence information at the site of hydrolysis is lost. Abasic sites have been chemically generated at random sites in DNA by acid[1] and/or heat treatment[1,2] however, placement of an apurinic/apyrimidinic site at a specific location has typically required the use of enzymes[3,4]. The 2'-deoxyribose residue which remains after glycosidic bond hydrolysis exists in a ring-opened (aldehyde) and ring-closed (furanose) tautomeric equilibrium with the former species highly base labile. Cleavage of the sugar-phosphate backbone at such sites readily occurs via β-elimination reactions. Owing to this instability, the chemical synthesis of DNA fragments containing abasic sites has not been previously reported. However, the recent interest in the structure and properties of apurinic/apyrimidinic sites has stimulated the synthesis of a number cyclic and acyclic base stable analogues which mimic the structure of the abasic site[5-11]. We wish to report that 2-pyrimidinone-containing oligodeoxynucleotides undergo facile acid-catalyzed hydrolysis. This property allows for the first time, a convenient chemical synthesis of DNA fragments which contain an abasic site at a preselected position.

During the course of the synthesis of 1-(β-D-2'-deoxyribosyl)-2-pyrimidinone (1 = dK)[12] and its 5-methyl derivative (2 = d5)[12] as analogues of dC and dT respectively, we observed that aqueous reactions which employed low pH conditions resulted in significantly reduced product yields primarily as a result of decomposition of the nucleoside. We examined this problem more closely and observed that the two nucleosides exhibited only limited stability in aqueous solutions at pH values below 5.0. Incubation of an aqueous solution of either nucleoside (1 or 2) at pH 4.0 or 3.0 [13] and ambient temperature resulted in a process which could be monitored by HPLC[14] (using UV detection). The product of the reaction was the intact 2-hydroxypyrimidine (from 1) or 2-hydroxy-5-methylpyrimidine (from 2). The identity of both products was confirmed by comparison with authentic standards. These results indicated that the observed decomposition involved hydrolysis of the N-glycosidic bond and release of the nucleobase. After a 24 h incubation at pH 3.0 the hydrolysis of 1 was >95% complete and that of 2 was >90% complete. Under the same conditions dC or dT exhibited little measurable hydrolysis (<1%) while minor amounts of the depurination products from dA and dG were observed.



1 2

Depurination was most significant for dG under these conditions but did not exceed 4% after 24 h. The glycosidic bond hydrolysis was pH dependent with an increased rate of hydrolysis at lower pH and was observed to follow pseudo first-order kinetics. The rate constants for the hydrolysis at pH 3.0 and ambient temperature were determined from the plot of Figure 1a to be 1.05×10^{-4} sec^{-1} and 1.44×10^{-4} sec^{-1} for 1 and 2 respectively.

Although the pyrimidinone nucleosides are labile to mild aqueous acid, the corresponding phosphoramidite derivatives can still be used as building blocks in routine (automated) syntheses commonly employed to construct DNA sequences[12]. Using the described procedures[12] two decamers, d(CTGAATTKAG) and d(CTGAAT5CAG), were prepared and subsequently incubated at pH 3.0 [13] (ambient temperature). HPLC analysis indicated that the peak corresponding to the dK or d5 oligodeoxynucleotide (~18 min) decreased during the incubation, and a new peak at ~16 min resulted. Nucleoside analysis of this new peak[15] indicated that the dK (1) or d5 (2) residue was absent while the ratios of the other nucleosides remained constant. The rate of hydrolysis of the dK and d5 residues incorporated within the oligodeoxynucleotides was clearly slower than that observed with the free nucleosides. Although a 60 h incubation was required for the hydrolysis to reach 90% completion significant amounts of the abasic product could be obtained after an overnight incuabation. In order to test whether the hydrolysis product contained the expected apurinic/apyrimidinic site, a sample of the reaction mixture was treated with piperidine under conditions known to accelerate chain cleavage at abasic sites.[16] Subsequent HPLC analyses indicated that rapid cleavage of the oligodeoxynucleotide ensued after the addition of piperidine, with the formation of two products, the expected result for chain cleavage (Fig. 2). Treatment of either the dK or d5 containing oligodeoxynucleotide with piperidine prior to incubation at pH 3.0 did not produce any cleavage products.

Fragments of 10 or 12 nucleotides in length and containing the abasic site could be easily resolved from the dK or d5 containing fragments using HPLC[17]. The ability to resolve the apurinic from the pyrimidinone containing oligodeoxynucleotide may limit the feasibility of this approach with longer DNA fragments. Although minor amounts of additional depurination of dG were expected under the desired conditions we could not observe these products. However, additional loss of guanine would result in a further decrease in retention time during the HPLC analysis and purification and would not have contaminated the isolated product.

Both hydrolytic reactions followed pseudo first-order kinetics and rate constants of 6.94×10^{-6} sec^{-1} and 8.61×10^{-6} sec^{-1} were obtained at pH 3.0 for d(CTGAATTKAG) and d(CTGAAT5CAG) respectively from the plot illustrated in Figure 1b.

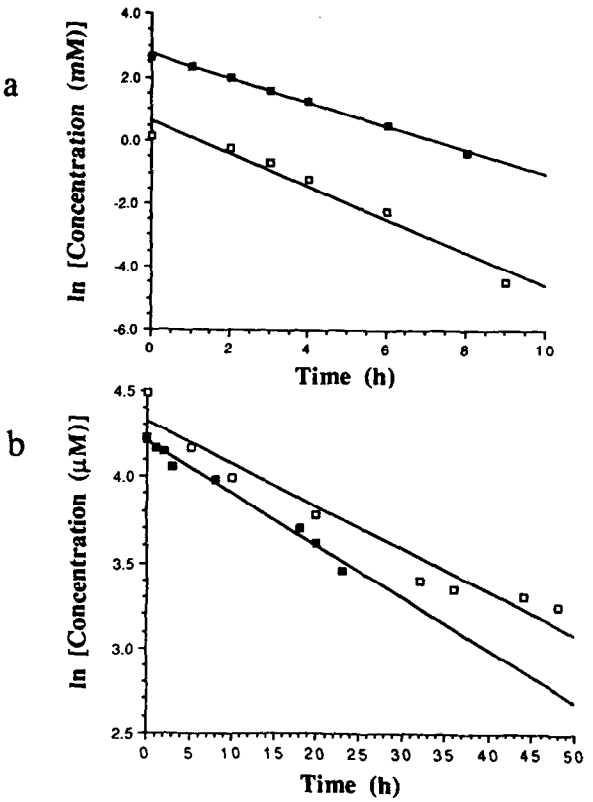


Figure 1. Kinetic plots of the hydrolysis of (a) dK (□) and d5 (■) at pH 3.0 and ambient temperature. (b) d(CTGAATTKAG) (□) and d(CTGAAT5CAG) (■) at pH 3.0 and ambient temperature.

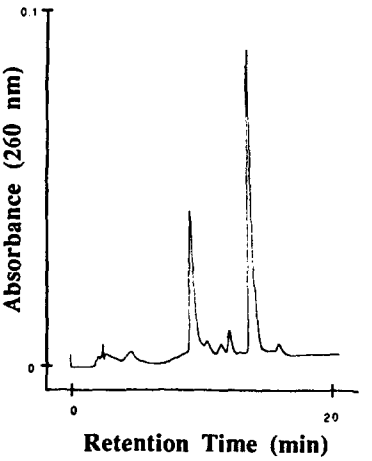


Figure 2. HPLC analysis of the decadeoxynucleotide d(CTGAATT_CAG) after treatment with piperidine. HPLC conditions: 4.6 x 250 mm MOS-Hypersil; Buffer: 20 mM KH_2PO_4 pH 5.5; Gradient: 0-35% methanol in 30 min.

We attempted to measure the Tm values for the dK and d5 fragments and corresponding hydrolysis products but found the duplexes too unstable to obtain reliable values. We then prepared two self-complementary dodecadeoxynucleotides, a native sequence: d(CGCGAATTCGCG) and a 2-pyrimidinone containing sequence: d(CGCGAATTKGCG). The corresponding fragment containing an apurinic site, d(CGCGAATT_GCG), was prepared by incubation of d(CGCGAATTKGCG) at pH 3.0 for 65 h at ambient temperature with subsequent isolation by HPLC[17]. Tm values of 61.7 °C, 42.3 °C and 20.3 °C were measured[18] for the fragments containing the native dC residue, dK residue and abasic site respectively. The greatly reduced stability of the helix containing two abasic sites[19] is a result of the missing hydrogen bonding and base stacking interactions important for helix stabilization. Although the observed decrease in stability in the present case might be exacerbated by the use of relatively short DNA fragments, the results suggest that significant local destabilization may result from the presence of an abasic site in longer DNA polymers.

Abasic sites are commonly formed in DNA by the spontaneous loss of a purine bases (depurination),[20] as a result of chemical agents,[21,22] and as intermediates during the normal course of repair mechanisms involving

glycosylases.[23,24] The study of the structure and properties of these sites will lead to a better understanding of the chemical (and biochemical) processes associated with nucleic acids.

In conclusion, mild aqueous acid catalyzes the cleavage of the N-glycosidic bond of 2-pyrimidinone nucleosides at rates much faster than observed for the four common 2'-deoxynucleosides. This was an unexpected and unusual result which we cannot at present adequately explain. This rate is reduced somewhat when the nucleoside residue is incorporated into a DNA fragment, but still provides a simple chemical procedure for the generation of abasic sites at specific locations within a given sequence.

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REFERENCES AND NOTES

1. Greer, S. and Zamenhof, *S. J. Mol. Biol.* **4**, 123-141 (1962).
2. Lindahl, T. and Nyberg, B. *Biochemistry* **11**, 3610-3617 (1972).
3. Manoharan, M., Ransom, S., Mazumder, A., Gerlt, J., Wilde, J., Withka, J., and Bolton, P. *J. Amer. Chem. Soc.* **110**, 1620-1622 (1988).
4. Stuart, G.R., and Chambers, R.W. *Nucl. Acids Res.* **15**, 7451-7462 (1987).
5. Millican, T.A., Mock, G.A., Chauncey, M.A., Patel, T.P., Eaton, M.A.W., Gunning, J., Cutbush, S.D., Neidle, S. and Mann, J. *Nucl. Acids Res.* **12**, 7435-7453 (1984).
6. Pochet, S., Huynh-Dinh, T., Neumann, J.M., Tran-Dinh, S., Adam, S., Taboury, J., Taillandier, E. and Igolen, J. *Nucl. Acids Res.* **14**, 1107-1126 (1986).
7. Takeshita, M., Chang, C.-N., Johnson, F., Will, S. and Grollman, A.P. *J. Biol. Chem.* **262**, 10171-10179 (1987).
8. Eritja, R., Walker, P., Randall, S., Goodman, M. and Kaplan, B. *Nucleosides and Nucleotides* **6**, 803-814 (1987).
9. Seela, F. and Kaiser, K. *Nucl. Acids Res.* **15**, 3113-3129 (1987).
10. Cuniase, Ph., Sowers, L.C., Eritja, R., Kaplan, B., Goodman, M.F., Cognet, J.A.H., Lebret, M., Guschlbauer, W., and Fazakerley, G.V. *Nucl. Acids Res.* **15**, 8003-8020 (1987).
11. Kalnik, M.W., Chang, C-N., Grollman, A.P., and Patel, D.J. *Biochemistry* **27**, 924-931 (1988).
12. Gildea, B. and McLaughlin, L.W. *Nucl. Acids Res.* **17**, 2261-2281 (1989).
13. 20 mM formic acid/sodium formate pH 3.0.
14. This analysis used a 4.6 x 250 mm column of MOS-Hypersil (C8-Silica); Buffer: 20 mM KH_2PO_4 pH 5.5; Gradient: 0-70% methanol in 30 min.
15. The oligodeoxynucleotides were digested with snake venom phosphodiesterase and bacterial alkaline phosphatase at 37 °C for 18 h. The nucleoside mixture was analyzed as described in note 19 using a 60 min gradient. Under these conditions 1 eluted at 10.1 min and 2 at 13.5 min (both exhibit a λ_{max} of 313 nm).
16. Maxim A. and Gilbert, W. *Methods Enzymol.* **65**, 499-580 (1980).
17. The isolation employed conditions as described in note 14 using a 60 min gradient. The dK containing dodecamer eluted at 14.6 min and that containing the abasic site at 12.5 min.
18. Tm values were obtained in 20 mM phosphate, 1.0 M NaCl, pH 7.0.
19. The duplex is formed from two self-complementary fragments each containing an abasic site.
20. For a review see: Singer, B. and Grunberger, D. "Molecular Biology of Mutagens and Carcinogens", Plenum, New York (1983).
21. Drinkwater, N.R., Miller, E.C. and Miller, J.A. *Biochemistry* 19, 5087-5092 (1980)
22. Loeb, L. and Preston, B. *Annual Review of Genetics* **20**, 201-30 (1986).
23. Lindahl, T. in "Progress in Nucleic Acid Research and Molecular Biology" **22** 135-192 (1979).
24. Foster, P.L. and Davis, E.F. *Proc. Natl. Acad. Sci. USA* **84**, 2891-2895 (1987).

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