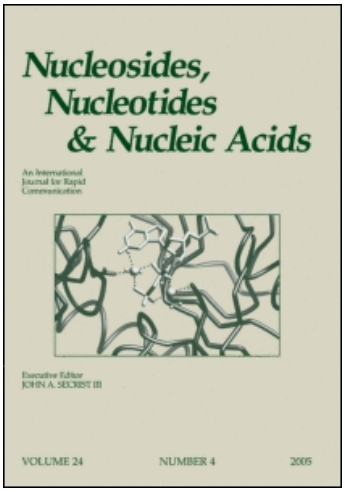

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Synthesis and Characterization of Oligodeoxyribonucleotides Containing Tandem Base Damage

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SYNTHESIS AND CHARACTERIZATION OF OLIGODEOXYRIBONUCLEOTIDES CONTAINING TANDEM BASE DAMAGE

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ABSTRACT : Both N(2-deoxy-β-D-*erythro*-pentofuranosyl)-formylamine (dβF) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) were introduced in synthetic oligonucleotides at a vicinal position via the solid phase phosphoramidite method in order to investigate the biological and structural significance of such a tandem lesion. Further experiments aimed at determining the enzymatic repair by both *E. coli* endonuclease III (Endo III) and Fapy-glycosylase (Fpg) were carried out with these synthetic substrates.

Several studies have shown that ionizing radiation can generate various lesions including base modifications, abasic sites, DNA strand breaks and tandem base damage [1]. The latter type of damage which may arise from one radical event is suspected to have deleterious biological effects. One example of tandem base damage induced by · OH radical reaction in oligodeoxynucleotides is the N(2-deoxy-β-D-*erythro*-pentofuranosyl)-formylamine (dβF) / 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) [2]. In order to investigate the biological consequence of such a tandem base lesion, both 8-oxo-7,8-dihydroguanine and formylamine, which have been already isolated from DNA, were introduced in synthetic oligonucleotides.

First, the preparation of the phosphoramidite synthon of 8-oxo-dGuo was performed using a new methodology. The nucleoside dβF was synthesized using a previous described procedure [3]. Then, stability studies of the formylamine lesion were carried out. It was found that the stability of dβF was compatible with the "Pac phosphoramidite" chemistry [4]. Then, dβF/8-oxo-dGuo containing oligonucleotides, which were constructed using the phosphoramidite chemistry on solid support, were obtained in good yields. The purity of the synthetic DNA fragments was confirmed using different complementary techniques: HPLC, polyacrylamide gel electrophoresis, electrospray and MALDI-TOF mass spectrometry. The integrity of modified nucleosides incorporated into oligonucleotides was assessed by HPLC and capillary electrophoresis analysis of the enzymatic digestion mixtures. The insertion of the tandem base lesion at defined sites was confirmed by a sequence analysis involving snake venom phosphodiesterase and calf spleen phosphodiesterase digestions in combination with MALDI-TOF measurement [5]. The piperidine test applied to the dβF/8-oxo-dGuo containing oligonucleotides showed the high lability of formylamine in DNA. Then, the enzymatic repair studies revealed that both Endo III and Fpg induce a nick in the oligonucleotides containing the tandem or the single dβF lesions. In addition, MALDI-TOF analysis of the generated fragments indicated that the mechanism of action of Endo III on the oligonucleotides is dependant on the lesions inserted. The major reaction observed is the cleavage of the phosphodiester bond 3' to the formylamine via an hydrolysis step. In addition, a β-elimination reaction was found to occur as minor pathway. Surprisingly, the Fpg protein was found to mainly excise the 8-oxo-7,8-dihydroguanine residue from the oligonucleotides that contain the tandem base damage, whereas the enzyme is able to remove a single formylamine inserted in a oligonucleotide.

REFERENCES

[1] a) Ward, J.F. Int. J. Radiat. Biol. 1994, 66, 427-432. b) Goodhead, D.T. Int. J. Radiat. Biol. 1994, 65, 7-17. c) Cadet, J.; Berger, M.; Douki, T.; Ravanat, J.-L. Rev. Physiol. Biochem. Pharmacol. 1997, 131, 1-87.

[2] Box, H.; Freund, H.; Budzinski, E., Wallace, J.; Maccubbin, A. Radiat. Res. 1995 141, 91-94.

[3] Guy, A.; Duplaa, A.M.; Ulrich, J.; Teoule, R. Nucleic Acids Res. 1991, 19, 5815-5820.

[4] Schulhof, J.C.; Molko, D.; Teoule, R. Nucleic Acids Res. 1987, 15, 397-415.

[5] Pieles, U.; Zurcher, W.; Schar, M.; Moser, H.E. Nucleic Acids Res. 1993, 21, 3191-3196.