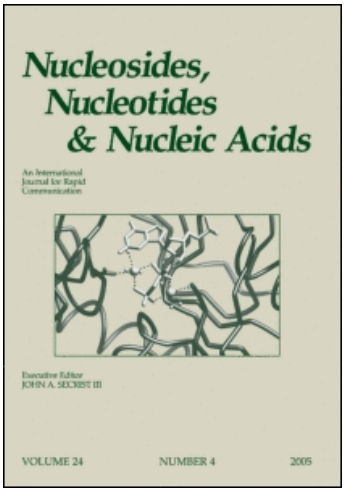

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Recognition of Cyclonucleoside Lesions by the *Lactococcus lactis* FPG Protein

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ABSTRACT

Several purine and pyrimidine cyclonucleosides were found to be not recognized by several *Escherichia coli* and yeast DNA *N*-glycosylases. Interestingly, a non covalent complex was observed between the *Lactoccocus lactis* formamido-pyrimidine-DNA glycosylases (Fpg-*Ll*) and the cyclonucleosides. This may provide new information on the mechanism involved in the activity of the latter enzyme.

Carbon-bridged cyclonucleosides were found to be generated in purine and pyrimidine nucleic acid monomeric components and in DNA polymers upon exposure to ionizing radiations.[1,2] To investigate the biological and structural effects of the

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formation of such anhydronucleosides, we have synthesized ODNs containing each of the 5′*R* and 5′*S* diastereoisomers of 5′,8-cyclo-2′-deoxyadenosine[3] and 5′,8-cyclo-2′-deoxyguanosine,[4] the 5*R* and 5*S* diastereoisomers of (5′*S*,6*S*)-5′,6-cyclo-5,6-dihydrothymidine,[5] (5′*S*,6*S*)-cyclo-5,6-dihydro-2′-deoxyuridine[6] and (5′*S*,5*S*,6*S*)-5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine.[7] *In vitro* replication studies and repair experiments have shown that the latter modifications act as blocking lesions for DNA polymerases and are not recognized by several DNA *N*-glycosylases, including formamidopyrimidine DNA glycosylase (*E. coli* Fpg), endo III and endo VIII of *E. coli*, Ntg1, Ntg2 and Ogg1 of *S. cerevisiae*.[6–8] Therefore, the cyclonucleosides may represent potential lethal lesions within the cell if they are not removed by the nucleotide excision repair machinery.

We report here the results of a comparative study on the behavior of *E. coli* Fpg and Fpg-*Ll* toward the above cyclonucleosides. *E. coli* Fpg have been well described in the literature. The latter DNA glycosylase was found to excise a wide range of oxidized bases[9] and to exhibit an AP lyase and endonuclease activity.[10] Recently, the *fpg-Ll* gene has been cloned and characterized.[11] The deduced sequence showed 59% similarity and 38% identity with the *E. coli* Fpg gena. The protein displays two enzymatic activities: a base excision and subsequent DNA nicking at the resulting abasic site. Recently, the binding of the Fpg-*Ll* protein to DNA fragments that contained either cyclic or non-cyclic abasic site analogs was investigated.[12] This provided new information on the molecular mechanism involved in the AP lyase activity of Fpg. The structure of a non-covalent complex between the Fpg-*Ll* protein and the 1,3-propanediol abasic site analog-containing DNA has been recently solved.[13] This highlights the Fpg and DNA structural features that are required to form a stable and non-covalent specific complex prior to the Schiff base formation.

The binding of the Fpg-*Ll* protein to DNA that contained the above cyclonucleosides was investigated using the electrophoretic mobility shift assay (Fig. 1).

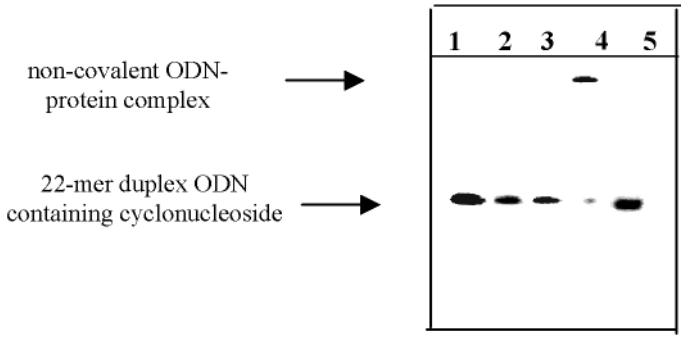

Figure 1. Non denaturing PAGE (15%) analysis of the enzymatic reactions of the 22-mer duplex containing the (5′*S*,6*S*)-cyclodHdUrd/dGuo base pair with Fpg-*Ll* and Fpg *E. coli* enzyme respectively. Lane 1: double stranded 22-mer oligonucleotide (5′*S*,6*S*)-cyclodHdUrd/dGuo. After treatment with: lane 2: *E. coli* Fpg (1000 ng); lane 3: *E. coli* Fpg (1000 ng) then with piperidine 1 M, 90°C 5 min; lane 4: Fpg-*Ll* (1000 ng); lane 5: Fpg-*Ll* (1000 ng) then with piperidine 1 M, 90°C 5 min.

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We observed the disappearance of the initial 22-mer duplex and the formation of a higher molecular complex when the 22-mer duplex containing any of the cyclonucleosides was incubated with the Fpg-*Ll* protein. The latter band disappears after a treatment with piperidine 1 M at 90°C that leads the restoration of the original signal at the 22-mer position. This was not observed when the duplex was incubated in the presence of the *E. coli* protein. The results strongly suggest that the Fpg-*Ll* protein is able to recognize but not to excise the cyclonucleoside derivatives, by forming a non-covalent complex. Interestingly, the *E. coli* Fpg protein is unable to recognize the cyclonucleosides lesions. It should be added that the unmodified 22-mer duplex was not recognized nor excised by the Fpg-Ll protein, whereas the protein was able to excise the 22-mer oligonucleotide that contained 8-oxodGuo (data not shown). Further investigations showed that the duplex is necessary for the complex formation (data not shown). Finally, the non covalent oligonucleotide-enzyme complex was observed either on a denaturing or non denaturing PAGE, in agreement with the formation of a stable complex.

The latter DNA/protein interaction reported here can be further used to gain new insights on the structural features and the molecular mechanisms involved in the Fpg protein activity, including the structure of the non-covalent complex and the design of site-directed mutagenesis experiments of the latter DNA glycosylase.[14,15]

REFERENCES

1. Cadet, J.; Berger, M. Int. J. Radiat. Biol. **1985**, *47*, 127–143.
2. Shaw, A.A.; Cadet, J. Int. J. Radiat. Biol. **1986**, *54*, 987–997.
3. Romieu, A.; Gasparutto, D.; Molko, D.; Cadet, J. J. Org. Chem. **1998**, *63*, 5245–5249.
4. Romieu, A.; Gasparutto, D.; Cadet, J. Chem. Res. Toxicol. **1999**, *12*, 412–421.
5. Romieu, A.; Gasparutto, D.; Cadet, J. J. Perkin Trans. I **1999**, 1257–1263.
6. Muller, E.; Gasparutto, D.; Jacquinod, M.; Romieu, A.; Cadet, J. Tetrahedron **2000**, *56*, 8689–8701.
7. Muller, E.; Gasparutto, D.; Cadet, J. Chem. Bio. Chem. **2002**, *3*, 534–542.
8. Gasparutto, D.; Bourdat, A.G.; D′Ham, C.; Duarte, V.; Romieu, A.; Cadet, J. Biochimie **2000**, *82*, 19–24.
9. David, S.S.; William, S.D. Chem. Rev. **1998**, *98*, 1221–1261.
10. Bhagwat, M.; Gerlt, J.A. Biochemistry **1996**, *35*, 659–665.
11. Duwat, P.; de Oliveira, R.; Ehrlich, S.D.; Boiteux, S. Microbiology **1995**, *141*, 411–417.
12. Castaing, B.; Fourrey, J.L.; Hervouet, N.; Thomas, M.; Boiteux, S.; Zelwer, C. Nucleic Acids Res. **1999**, *15*, 608–615.
13. Serre, L.; Pereira de Jésus, K.; Boiteux, S.; Zelwer, C.; Castaing, B. EMBO J. **2002**, *21*, 2854–2865.
14. Zharkov, O.; Grollman, A. Free Radical Biol. Med. **2002**, *12*, 1254–1263.
15. Boiteux, S.; Gellon, L.; Guibourt, N. Free Radical Biol. Med. **2002**, *12*, 1244–1253.

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