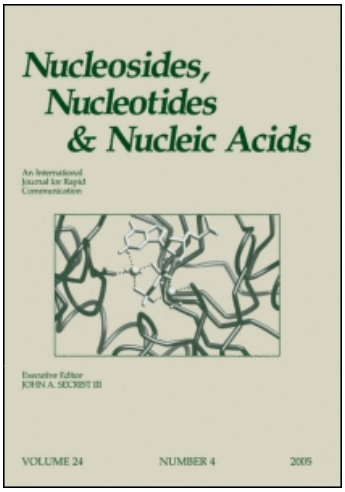


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# Cross-Linking of *Escherichia coli* Formamidopyrymidine-DNA Glycosylase to DNA Duplexes Containing Photoactivatable Phenyl(Trifluoromethyl)diazirine Groups

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# Cross-Linking of *Escherichia coli* Formamidopyrymidine-DNA Glycosylase to DNA Duplexes Containing Photoactivatable Phenyl(Trifluoromethyl)diazirine Groups

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## ABSTRACT

New reactive analogs of substrates for DNA repair enzyme *E. coli* Fpg protein containing the residues of 8-oxoguanine and photoactivatable phenyl(trifluoromethyl)diazirine groups were synthesized. Their substrate properties were investigated. Using photocross-linking technique, we established the presence of contacts of two nucleosides located near the oxoG with amino acids from the Fpg protein. The cross-linking efficiency achieved 10%.

Fpg protein is a DNA repair enzyme that catalyzes the removal of oxidized purine bases, most notably the mutagenic 8-oxoguanine (oxoG) lesion from DNA, and cleaves DNA strand.[1] To ascertain specific contacts between amino acids from the Fpg protein and nucleosides closed to the oxoG lesion, modified DNA duplexes containing simultaneously the residues of the oxoG and 5-[4-[3-(trifluoromethyl)-3H-

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diazirin-3yl]phenyl]-2′-deoxyuridine (dU*) were synthesized:

5′ AgCTTgCATgCCdU*(oxoG)AggTCgACTCTAgAg (I)
gAACgTACgg-A--------C-TCCAgCTgAg

AgCTTgCATgCC-T(oxoG)--AggTCgACTCTAgAg (II)
GAACgTACggA-----CdU*CCAgCTgAg

The residue of dU* bearing photoactivatable 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenyl (TFMDPh) group was introduced in the oxoG-containing strand of 29/22-membered DNA duplex 5′ adjacent to the oxoG (duplex I) or in opposite strand 5′ adjacent to the cytidine forming base pair with the oxoG (duplex II). We found that Fpg protein recognizes and specifically binds DNA duplexes I and II with high efficiency. Our results indicate that the introduction of TFMDPh group in close proximity to the oxoG residue did not influence on the recognition and binding of DNA duplexes by the Fpg protein. To study the substrate properties of modified DNA duplexes I and II, their catalytic incision by the Fpg protein was investigated. It was revealed that DNA duplexes I and II are cleaved by the enzyme. The efficiency of DNA incision was depended on the position of TFMDPh group and was higher for DNA duplex I carrying this reactive group 5′ adjacent to the oxoG residue. In order to ascertain specific contacts between Fpg protein and nucleosides closed to the oxoG we have used a photocross-linking procedure. Specific complexes of the Fpg protein with radiolabeled DNA duplexes I and II were UV-irradiated (wavelength 366 nm) for 30 min on ice using a high intensity UV lamp. As shown in the Fig. 1, the cross-linking products were observed in both cases. Cross-linking should to be specific because the binding of the Fpg protein to DNA duplexes I and II resulted in only one specific DNA-Fpg protein complex. Cross-linking efficiency was as high as 10% for DNA duplex I containing photoactivatable group 5′ adjacent to the oxoG, and 2% for DNA duplex II. This distinction can be explained by the different accessibility of the amino acids to the reactive TFMDPH groups and, in less extent, by the different nature of the amino acids. The results obtained together with the ongoing studies of the Fpg protein and its pro- and eucaryote homologs will further elucidate the molecular mechanism of DNA repair. The approaches developed can be employed in the studies of others DNA repair enzymes.

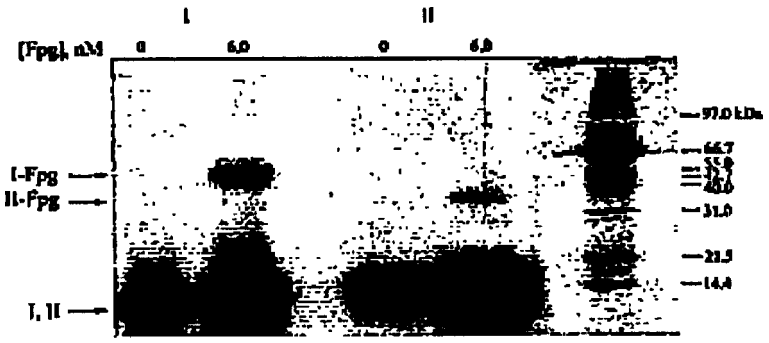


*Figure 1.*





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