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SEQUENCE SPECIFICITY IN PSORALEN-DNA PHOTOBINDING

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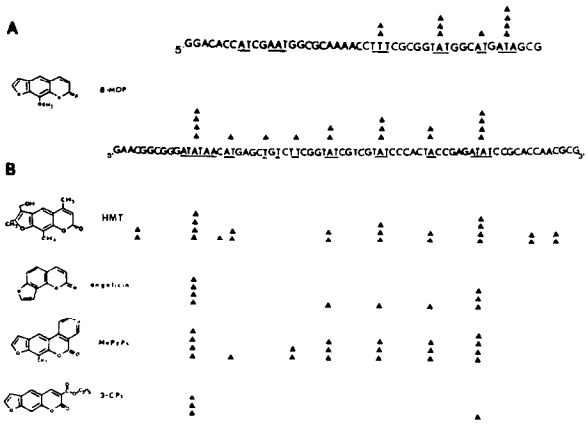
Furocoumarin DNA photoadducts provide an attractive model to study the influence of DNA sequence on mutagenesis and repair. The photoreaction of furocoumarins with DNA is a multistep reaction (1). The initial step is the intercalation of the psoralen within the double helix. Upon UVA irradiation (320-400 nm) cycloaddition occurs to the 5,6 double bond of pyrimidine bases (mainly thymine) through the pyrone or the furan ring of the psoralen molecule. The furan-side monoadduct can absorb a second photon and, when properly located, be converted to a diadduct which yields an interstrand crosslink, in the case of bifunctional compounds. The effect of adducts on the local structure of the DNA depends on the psoralen derivatives.

We report the mapping and quantitation of photoadducts induced by several psoralen derivatives in DNA fragments of defined sequence. The furocoumarins used are the bifunctional compounds 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), 4'-(hydroxymethyl)-4,5',8 trimethylpsoralen (HMT) and monofunctional compounds angelicin, 3-carbethoxypsoralen (3-CPs), pyridopsoralens (MePyPs, PyPs, BCH 394). The substrates are two DNA fragments of the lacI gene of E. coli, where mutations induced by 8-MOP and angelicin have been mapped. 5'-end-labeled DNA is incubated in presence of an excess of furocoumarin ($2.5\text{-}5 \times 10^{-4}$ M) and irradiated with doses of 365 nm light ranging from 6 to 72 KJm^{-2} and yielding to about 1 adduct per DNA fragment. The sites of photoaddition are analyzed by DNA sequencing methodology. They are revealed after digestion of modified DNA with the 3'-5' exonuclease associated to the T4 DNA polymerase, followed by electrophoresis of hydrolysis products on sequencing gel. Bulky 8-MOP-monoadducts, as well as biadducts, block the 3'-5' exonuclease activity (2). When using bifunctional compounds, crosslinks are photoreversed by a dose of 6 KJm^{-2} of 254 nm radiation, after the enzymatic treatment. We determine the relative frequency of adduct formation at each site by measuring radioactivity in each band. Maps of adducts distribution along two DNA fragments are established for several psoralen derivatives.

Autoradiograms reveal that the exonuclease terminates its digestion at the site of monoadduct and one nucleotide before a base involved in a biaddition. Moreover, each site gives rise to several termination events (at least two for sites like AT or TA). This is likely to reflect the presence of the different types of monoadducts.



Legend to the figure :

Nucleotide sequences of DNA fragments showing relative frequencies of photoadditions. The sites are scored as very strong (4▲), strong (3▲), medium (▲▲) and weak (▲).

By comparing the different sites of termination events for each compound, the DNA sequence selectivity in the photobinding can be deduced. Typically for 8-MOP photoreaction (2), thymine in a GC context is very poorly reactive ; adjacent thymines constitute a better target. The photoreaction occurs preferentially in potentially crosslinkable sites, and much more specifically in 5'-TpA versus 5'-ApT, in agreement with Tessman et al. (3). Crosslinks are formed exclusively at 5'-TpA sites. Furthermore, the difference in the reactivity of several TAT sites in the DNA fragments demonstrates the influence of the sequence context on the photobinding of 8-MOP to DNA. An increment in the reactivity of multiple crosslinkable sites is observed. Repeated $(AT)_n$ sequences are hotspots for the 8-MOP photobinding.

Strikingly, a similar overall picture in the spectrum of photoaddition of different psoralen derivatives is observed. This is illustrated in the figure which represents semi-quantitative maps of the photoaddition of several compounds along one DNA fragment. The same specificity is found. It means that the photoreaction of psoralen with DNA follows a general rule : there is a preference for 5'-TpA sites and repeated $(AT)_n$ sequences.

Nevertheless, some differences appear. HMT, a compound known to photoreact with cytosine residues (4), produces termination events at crosslinkable 5'-CpA or 5'-ApC sites. 3-CPs which has a low affinity for DNA, photoreacts exclusively with AT-rich sequences. In contrast, pyridopsoralens which have a high affinity for DNA, photoreact substantially with adjacent thymines. The observation that thymine residues are not equally reactive leads us to define two types of target sites. The "strong sites", i.e. $(AT)_n$ sequences, the most frequent, are preferential targets for all psoralen derivatives. In contrast, "weak sites", i.e. T or TT in a GC context, are targets for psoralen compounds having a high affinity for DNA.

In conclusion, our work highlights the role of the sequence context and of the local conformation in the photoreaction of psoralens with DNA. It allows us to predict the sites of photoadduct formation in any genomic DNA.

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