Biophysical Aspects of Auger Processes, AAPM Symposium Series No. 8 (1992) 153-163
Eds. RW Howell, VR Narra, KSR Sastry, DV Rao

^{125}I DECAY IN SYNTHETIC OLIGODEOXYNUCLEOTIDES

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ABSTRACT

The availability of synthetic oligodeoxynucleotides provides the opportunity to study the effects of ^{125}I in DNA with added precision and detail, compared to an earlier study (Martin and Haseltine, *Science* **214**, 296, 1981). We have designed a template/primer system which enables incorporation of ^{125}IdC into a defined location in a 31mer. The main advantage of this approach is that relatively large amounts (a few μCi) of DNA with both ^{32}P and ^{125}I can be produced quite easily. Consequently, when ^{125}I-induced DNA damage is analyzed on DNA sequencing gels, the distribution of ^{32}P-labeled cleavage products can be determined more accurately, following accumulation of decay events under various conditions (*e.g.* temperature, buffer components, free radical scavengers). The results of preliminary experiments suggest that the yield of DNA strand breaks per decay approaches one, at least for the ^{125}I labeled strand. Extension of this experimental approach will also enable investigation of the nature of the terminii (*e.g.* phophoryl - *versus* phophorylglycollate) at either side of ^{125}I decay-induced DNA strand breaks. The question of energy migration is also being investigated in the oligodeoxynucleotide system, by studying the effects of inclusion of BrUdR nucleotides at various distances from the ^{125}IdC.

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INTRODUCTION

The biological effectiveness of ^{125}I decay in DNA stems from the intensely focussed radiochemical damage that arises from two successive Auger cascades. The appreciation of the microdosimetry involved was underlined in studies involving Monte Carlo analysis of the ^{125}I decay event (1), and track structure analysis of each of the electrons emitted in simulated decays (2). The molecular analysis of DNA damage resulting from DNA-associated ^{125}I decay began with quantitation of strand breakage by sedimentation analysis of DNA from bacteriophages (3,4), *E. coli* (5) and eukaryote cells (6), in which ^{125}I decays had been accumulated. From such studies emerged the finding that on average, each ^{125}I decay yielded approximately 1 DNA double strand break.

The advent of restriction endonucleases and DNA sequencing techniques provided the opportunity to produce DNA molecules with a single ^{125}I atom in a defined location, and to analyze the location of DNA strand breaks resulting from accumulated ^{125}I decay events. These studies showed that DNA-associated ^{125}I decay resulted in the induction of single-strand breaks in both ^{125}I-containing strand, and the opposite strand. Most of this strand breakage occurred within four to five base pairs of the ^{125}I-labeled nucleotide (7).

These two approaches, microdosimetry and DNA damage analysis, eventually converged in an important study in which the energy deposition patterns from simulated ^{125}I decays were superimposed on a model of the B-DNA helix (8). This study actually utilized the distribution of DNA damage reported from the DNA sequencing study (7). In fact one of the parameters of the microdosimetry model, namely the amount of energy required to be deposited in a DNA strand to yield a single-strand break, was set at a value of 17.5 eV to give optimal concurrence of the model with the experimental data from DNA sequencing analysis. These studies, which were later refined (9), enabled an extension of this microdosimetric approach to other forms of radiation (10). More importantly, in the current context, the studies provided an explanation for the observation that the number of ^{125}I decays per lethal event in the eukaryote genome could be as high as 100. The potential explanation for this resides in the fact that the dosimetric studies demonstrated a wide variation amongst different decay events, in the nature

of the ^{125}I decay induced DNA double strand breaks. In short, some DNA double stranded breaks involved a lot more extensive damage, than others.

Regardless of the relationship between ^{125}I decays and lethal events, all the above studies, when taken together, indicated a picture in which most of the induced DNA double stranded breaks were in the close vicinity to the site of ^{125}I decay in DNA. This view was contradicted by an electron-microscopy study which reported strand breakage at sites distant from ^{125}I decay (11). Furthermore, there have been a number of proposals for energy transfer in DNA (12,13). These ideas have underlined the fact that in the earlier DNA sequencing studies (7), the efficiency of strand breaks induced per decay, was not actually measured, due to limitations in the technology available at that time. The assumption that the one double strand break per ^{125}I decay refers to a DNA double strand breaks induced at the site of decay, is clearly an important issue to verify. In the earlier studies of measuring DNA double strand induction per ^{125}I decay event (3-6), the observation of an average of about one DNA double strand break per decay was not inconsistent with the idea that not all these breaks are at the site of ^{125}I decay. The availability of the means to easily produce synthetic oligodeoxynucleotides provides the opportunity to revisit the application of DNA sequencing techniques to study DNA damage from ^{125}I decay. In particular it provides a possibility of measuring the absolute efficiency of strand break induction in the vicinity of the ^{125}I decay event. This communication reports our progress in this endeavor.

MATERIALS AND METHODS

The design strategy for the experiments is illustrated in Fig. 1. Briefly, oligodeoxynucleotides are synthesized (Applied Biosystems, Model 26A), and annealed to yield a template/primer configuration. Hybridization was monitored by electrophoresis in native polyacrylamide gels, after 5'-^{32}P-end labeling (4) of the primer oligoDNA. Carrier-free ^{125}I-dCTP (NEN-Dupont) is then incorporated by T4 DNA polymerase (Promega), and the primer extension completed by the addition of excess dCTP and other dNTPs. The extended primer strand is then isolated from a (denaturing) DNA sequencing gel, by excision of a gel slice and extraction in the presence of excess 31mer template strand. The extracted, full length "target" DNA, migrates as double-stranded hybrid on native polyacrylamide gel electrophoresis. Minor

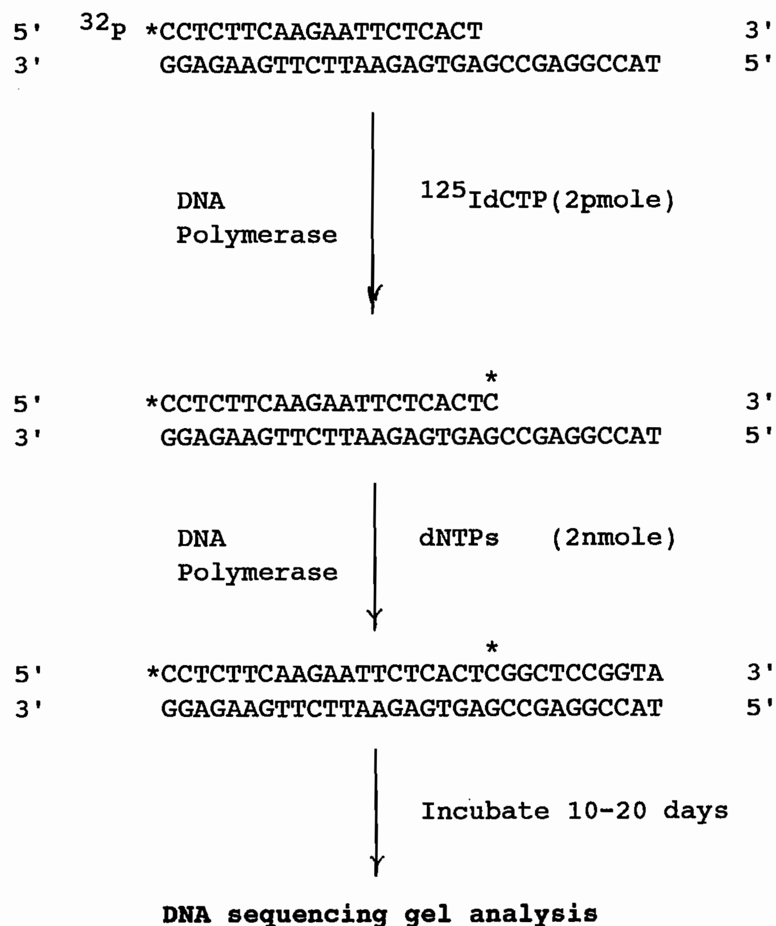

FIG. 1. ^{125}I decay in oligoDNA; outline of basic experimental design.

modifications to this strategy allow labeling of the ^{125}I-containing strand at the 3'-end, and then opposite (template) strand at either end.

At this juncture it is worthwhile to note the flexibility of the design strategy. For example, Br-dUTP can be included in the dNTP mixture. This enables investigation of energy transfer, as ^{125}I decay-induced strand cleavage adjacent to the Br-U sites. This feature is illustrated in Fig. 2. Furthermore, it will be noted that the target DNA in Figs. 1 & 2 comprises a binding site (AATT) for Hoechst 33258 and similar analogs. This provides the opportunity

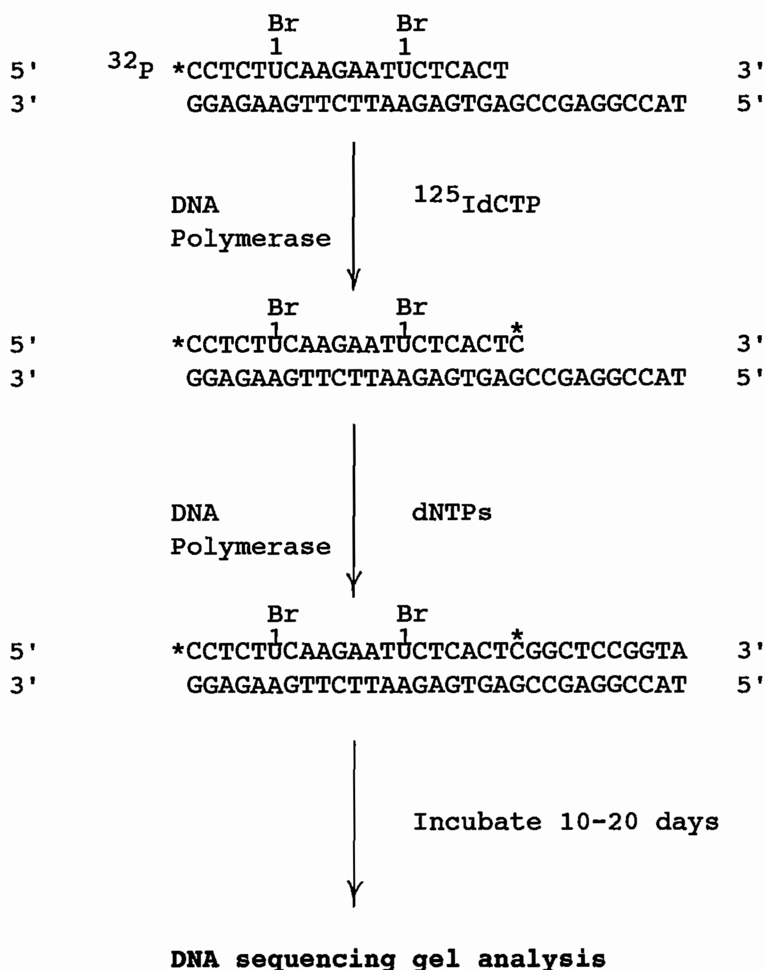

FIG. 2. ^{125}I decay in oligoDNA; experimental design to investigate energy transfer (*i.e.* induction of strand breaks at BrU sites, by ^{125}I-decay.

to investigate the effects of these radioprotecting ligands (15) on ^{125}I-induced DNA strand breakage. A further opportunity presented by this strategy is to use the DNA sequencing technology to investigate the nature of the terminii produced by ^{125}I-decay-induced DNA-strand breaks. Irradiation of 5'-end labeled DNA reveals the presence of doublets in DNA sequencing gels, first described by Henner *et al.* (16). The slower of these doublets corresponds to 3'-phosphoryl terminii, and the faster, 3'-phosphorylglycollate (15,16). The latter

is indicative of strand breakage initiated by abstraction of 4'-deoxyribosyl C-Hs (17), but are not observed under conditions of direct action (18).

The use of short primers maximizes the resolution of the DNA sequencing gel analysis and hence of detection of 3'-phosphorylglycollate species. A strategy is outlined in Fig. 3. After synthesis and isolation of the ^{125}I-oligoDNA, the sample is allowed to accumulate ^{125}I- decays under various conditions, such as:

(i) 0.5 M NaCl in 25 mM Tris HCl (pH 7.5) /5 mM EDTA/2 mM KI, at 4°C or at -20°C.
(ii) as in (i) except that 10% glycerol is included.
(iii) as in (i) except that 100 mM mannitol is included.

Given the relative half-lives of ^{125}I and ^{32}P (the "indicator" isotope), the optimal storage time is 15-20 days. At this time, the samples were denatured and analyzed on DNA sequencing gels (14,16). When required, an X ray film autoradiograph was used to locate the ^{32}P-labeled DNA fragments, and the individual bands excised from the gel. In this preliminary study, the amount of ^{32}P in the product DNA bands, and the parent full-length bands, was quantitated by Cerenkov counting. The fraction of ^{32}P in fragment bands compared to the total of parent and fragment bands was then calculated, and taken to indicate the average numbers of breaks per parent molecule. This calculated value was then compared with the number of ^{125}I decays accumulated over the incubation period.

RESULTS

The results of one experiment are shown in Fig. 4. The autoradiograph shows the resolution obtained between successive bands of ^{125}I-decay induced fragments; sufficient for excission and quantitation of individual bands. Since the load of ^{32}P-DNA varies between the different lanes, it is not possible to compare the various storage conditions by direct inspection. Some preliminary results, obtained from gels such as that shown in Fig. 4, suggest that the yield of DNA strand breaks per decay approaches one, at least for the ^{125}I-labeled strand. More data is required to evaluate the effect of storage conditions. Moreover, the quantitation of ^{32}P in gel slices by Cerenkov counting has been found to be sub-optimal, and alternative methods (*viz* use of scintillants) are being investigated.

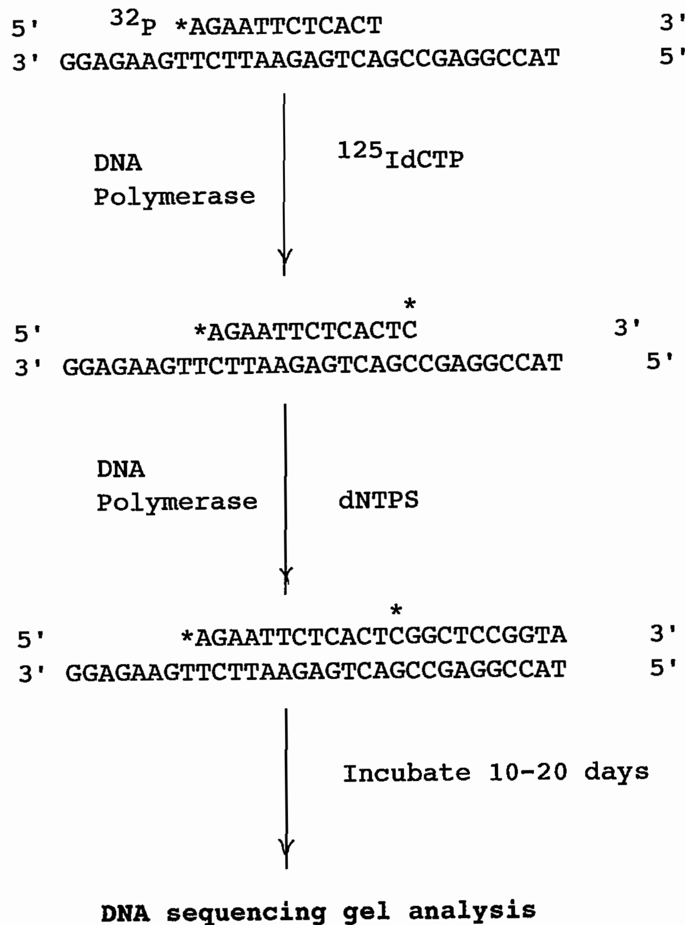

FIG. 3. ^{125}I-decay in oligoDNA; experimental design to investigate chemical nature of the terminii left by ^{125}I-decay-induced strand breaks. The short 5'-end-labeled primer maximizes the resolution obtained on DNA sequencing gel analysis.

DISCUSSION AND CONCLUSIONS

In order to evaluate the results reported here, it is important to appreciate the limitations of the earlier DNA sequencing study (7). That was done at a time when DNA sequencing was in its infancy, and obtaining sufficient ^{32}P-labeled material was a major problem. The ^{125}I-dCTP was

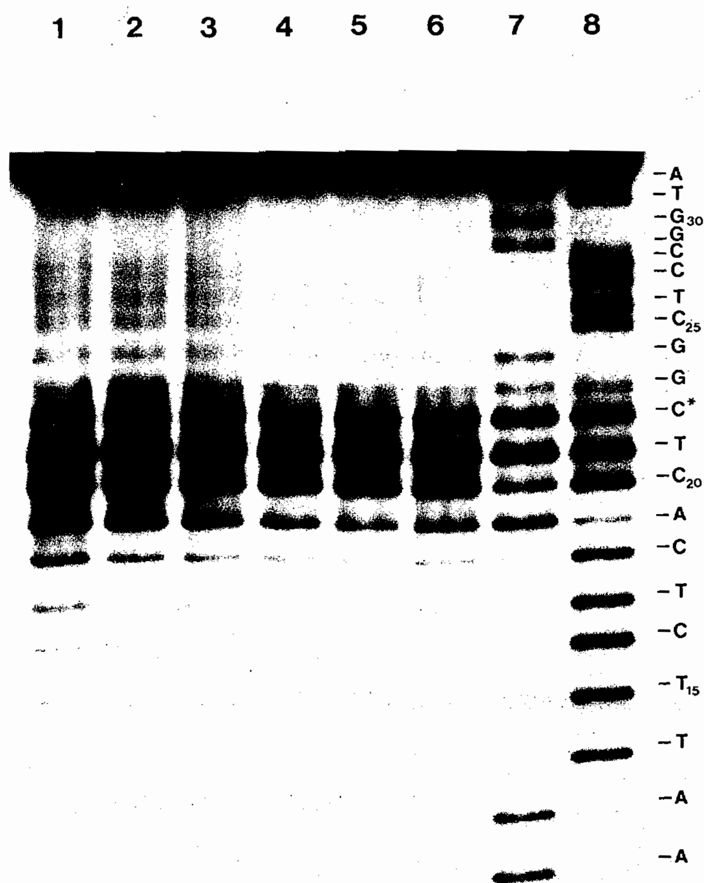

FIG. 4. DNA sequencing gel analysis of ^{125}I-decay-induced damage in oligoDNA. The ^{125}I/^{32}P double-labeled oligoDNA samples (Fig. 1), were stored for 12 days prior to DNA sequencing gel analysis, either at 4°C (lanes 1-3) or -20°C (lanes 4-6). Prior to storage, glycerol was added to the level of 10% in some samples (lanes 2 and 5), and mannitol to 100 mM in others (lanes 3 and 4). The asterisk in the sequence at right indicates the site of incorporation of ^{125}IdC.

introduced by 3'-end labeling of plasmid DNA restriction fragments, after 5'-^{32}P end labeling. The required labeled fragment was purified by preparative gel electrophoresis, denatured and reannealed to template strand. The procedure required successive rounds of preparative gel electrophoresis, and recovery of ^{32}P-DNA was generally low. Indeed it proved impossible to obtain

adequate yields of extended ^{125}I-labeled primer. When the appropriate ^{32}P/^{125}I double-labeled DNA species for the experiment were obtained, and then allowed to accumulate ^{125}I decays, the yield of the resulting ^{32}P-labeled fragments was only sufficient to yield images on X ray film after exposure for several days. In other words, there was not enough ^{32}P-labeled DNA to actually quantitate by counting gel slices. Densitometry was used to quantitate the relative yields of the ^{32}P-labeled fragmentation products. It was not possible to calculate the yields of DNA strand breaks per ^{125}I-decay. In contrast, the availability of synthetic oligoDNA enables preparation of the required double-labeled DNA in large amounts.

The potential of the approach is clear from the autoradiograph in Fig. 4. The bands are well separated and easily excised from the gel for counting. However, the results of the quantitation experiments must be taken as preliminary at this stage. The important conclusions from the results to date are that the use of ^{125}I-oligodeoxynucleotides should provide more precise data on the yield of strand breaks from ^{125}I decay in DNA, as well as information on the chemical nature of the terminii in ^{125}I-decay-induced DNA strand breaks, and the extent of energy transfer to BrU sites.

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DISCUSSION

Adelstein, S. J. Before we write off indirect effects (mediated by watery radicals) two matters should be taken into account: 1) Mannitol is a less than ideal OH radical scavenger, and 2) Although strand breaks may not be mediated through these radicals, base damage may be.

Martin, R. Yes, I agree that mannitol is not an ideal scavenger (in fact we have learned that by experience), and we plan to use others, but 100 mM mannitol in the frozen state should restrict "indirect" effects. The other relevant point, which I did allude to briefly, is the investigation of strand break terminii; 3-phosphorylglycollate terminii might be an indicator of indirect action.

Baverstock, K. Have you checked to see whether you find preferential strand breakage close to cold bromine in DNA when you use γ radiation?

Martin, R. No, but of course we plan to do such experiments.

Hofer, K. G. From your presentation I am not quite certain about the existence of energy transfer. Are you saying that energy transfer exists, does not exist, or you do not know?

Martin, R. Our preliminary results suggest that energy transfer is limited, on the basis of two points: 1) Since the yield of SSB at the site of decay seems to approach unity, there is no need to invoke energy transfer to explain the established experimental findings of an average of 1 DSB/decay (an observation of substantially less than 1 SSB/decay at the site of decay would have come to the opposite conclusion). 2) In our experiment, measuring energy transfer in terms of excitation of BrUdR (and hence strand cleavage) by ^{125}I decay we could not observe any evidence of transfer over a distance of 8 base pairs.