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PROGRESS REPORT

MASTER

To: United States Atomic Energy
Commission

Contractor: Brandeis University
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Title of Project: Molecular Basis for the Mutagenic and
Lethal Effects of Ultraviolet
Irradiation

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Background and Progress Report

During the past year, our laboratory has directed its activities toward the further elucidation of the enzyme mechanisms by which UV-damaged DNA is repaired. The following description of the various steps is outlined in Fig. 1 which provides for the excision repair cycle.

I. Incision

The incision step in repair can be specifically catalyzed by an endonuclease derived from Micrococcus luteus cells. This enzyme, purified to homogeneity by isoelectric focusing with a $M = 15,000$, makes a single-strand break on UV-irradiated DNA 5'- to the UV photoproducts. Its binding and hydrolytic properties are currently under investigation.

ϕ X 174 DNA is a single-stranded circular DNA which forms a circular duplex (RFI) as an intermediate in the life cycle of ϕ X174 infected on E. coli. This replicative form (RFI) has been isolated and purified from E. coli cells infected with ϕ X.174 in the presence of tritiated thymidine. ^3H -RFI is being used as a substrate for studying the binding and hydrolytic properties of the UV-endonuclease. The basis of the assay consists of the ability of double-stranded circular DNA to pass through a nitrocellulose filter. Specific interaction of RFI DNA with protein results in retention of the complex on the filter. Moreover, one can also measure the ability of such a protein to catalyze a single-strand break in such a DNA molecule. After reaction, the enzyme reaction is inactivated by heat which in turn denatures the circular DNA molecule to form, for each single-strand break, one single-strand circular DNA molecule and a linear single-stranded molecule, both of which adhere to nitrocellulose filters. Thus, it is possible to measure the requirements for binding of the enzyme to the DNA and after denaturation, to measure the binding of the single-stranded forms to such a filter if hydrolysis had in fact occurred. Using this sort of an assay system, we have been able to examine which conformational changes occurring on the DNA molecule are required for enzyme binding and also what conformational changes are required for enzyme binding and also what conformational changes are required for hydrolysis action by the enzyme.

This enzyme, which does not require divalent cation, is stimulated by magnesium. EDTA, however, supports binding of the enzyme to RFI circles. Magnesium is required for the hydrolytic step in which UV-irradiated RFI DNA is employed as a substrate. Under these circumstances, it is now possible to determine what structural changes are required in order for this enzyme to bind and to act hydrolytically.

II. Reaction with Formaldehyde

Studies at constant ionic strength with increasing temperatures between 0° and 37° followed by fixation in formaldehyde at 0° results in significant complexing with the enzyme and concomitant hydrolysis. Therefore, introduction of these conformational changes fixed by formaldehyde results in recognition sites specific for this enzyme. We are currently examining the effect of producing a single nucleotide modification in which cytosine is converted to uracil residues in the presence of sodium bisulphite. The binding constants of the enzyme have been measured as well as the equilibrium constants for the interaction of the enzyme with intact and with incised DNA.

III. Excision

The excision step is catalyzed by a specific exonuclease which has also been purified to homogeneity. This enzyme initiates hydrolysis from both 5' and 3' termini liberating 5'-nucleotides from denatured DNA. The appKm's have been determined in this initiation process using triply labelled poly dTs as a substrate in which the 5'-terminus contains 60 ³H-thymidylate residues followed by approximately 200 unlabelled thymidylate residues and at the 3' terminus are approximately 60 ¹⁴C-thymidylates. It has been determined, using such a substrate, that the apparent Km's for binding are six times lower at the 3' terminus than for such binding at the 5' terminus. The same sort of information is now being applied to determine the Km's for binding to the 5' terminus of incised regions of irradiated DNA.

IV. Reinsertion Mechanism

Prereinsertion--Based on the information obtained concerning the mechanism of action of the endonuclease and exonuclease with irradiated DNA, it is apparent that prior removal of the 3' phosphoryl group remaining on the excised DNA must be removed in order for polymerization to occur. It has been assumed that such a nucleophilic site can be revealed through the action of either aspecific polynucleotide phosphomonoesterase or an exonuclease III type enzyme which can catalyze a dephosphorylation prior to selective removal of nucleotides in a 3'-5' direction. The latter type enzyme activity has been found to be associated with the homogeneous DNA polymerase isolated from Micrococcus luteus. Thus, DNAs having 3' phosphoryl groups bordering the cavities in DNA, do not require prior dephosphorylation by an exogenous phosphomonoesterase in order for polymerization to occur. We have isolated a mutant of M. luteus which is extremely sensitive to ultraviolet light, is able to carry out normal excision, but is unable to reinsert nucleotides during a post-irradiation period in vivo. The DNA polymerase from this mutant has been isolated and purified to homogeneity which lacks the 3'-5' activity normally found in the wild

type enzyme. Thus, one of the nuclease activities normally associated with the DNA polymerase polypeptide is absent in the mutant and may account for its UV sensitivity.

Figure 1

Publications Supported by this Contract

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