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TECHNICAL PROGRESS REPORT

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AN IMMUNOCHEMICAL APPROACH TO THE STUDY OF DNA DAMAGE AND REPAIR

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TECHNICAL REPORT

The overall objective of this project is to produce antibodies to unique modified DNA bases and develop immunochemical assays to quantitate these lesions in damaged DNA. During this past year we have developed an antibody and chemical test to quantitate abasic sites in DNA and produced antibodies to the 8-oxopurines.

1. Detection of Abasic Sites in DNA

Abasic (apurinic, apyrimidinic, AP) sites are the most common cellular DNA lesion. Thus it seemed desirable to develop an immunochemical method to measure this important lesion. Our strategy consisted of eliciting an antibody specific for a reagent that reacts with the aldehyde group that is formed as a result of depurination of DNA. The reagent chosen was 0-4-nitrobenzylhydroxylamine. Monoclonal antibodies specific for this moiety were raised by immunizing with a conjugate of BSA and the 0-4-nitrobenzylhydroxylamine of deoxyribose-5'-phosphate. Screening was done by an ELISA using 0-4-nitrobenzylhydroxylamine deoxyribose-5'-phosphate conjugated to RSA. Of 40 wells tested, 10 were positive. The positive supernatants were subsequently assayed by ELISA for binding to depurinated calf thymus DNA (200 sites per 10,000 bp) treated with 0-4-nitrobenzylhydroxylamine. Of the 10 positive lines, one reacted with the apurinic-DNA, as well as with native calf thymus DNA, both treated with 0-4-nitrobenzylhydroxylamine. Two clones bound treated apurinic DNA only. The latter were subcloned twice by limiting dilution. Four clones were isolated and culture supernatants were collected. Ascites were prepared from them as well. Results of this work have been submitted to BIOCHEMISTRY.

We have used the same principle to develop a sensitive chemical assay for quantitating abasic sites. We have synthesized a chemical compound, biotin-conjugated O-carboxymethylhydroxylamine (aldehyde-reactive probe, ARP), that reacts specifically and quantitatively with the aldehyde group of abasic sites in DNA. Therefore, we can tag the abasic site in the DNA with biotin and then determine the number of biotin-tagged abasic sites using horseradish peroxidase-conjugated avidin-biotin complex in an ELISA-like assay.

The abasic site (ABS) test directly detects abasic sites in exposed DNA. This can be done in two experimental formats: 1) DNA can be directly exposed to the agent *in vitro* and the number of abasic sites produced determined. 2) An *E. coli* mutant, defective in AP endonucleases essential for base excision repair, can be exposed to an agent that produces AP sites using a suspension of the bacterial strain. Using this assay, we have been able to detect generation of abasic sites in *E. coli* cells produced by 0.5 g/ml methyl methanesulfonate (MMS). We calculate that the ABS test is able to detect 1 abasic site per 2 million bases on 100 nanograms of DNA. Results of this work will be submitted to PNAS.

2. Preparation of Antibodies to 8-Hydroxyadenine and 8-Hydroxyguanine

The deoxyribose derivatives of 8-hydroxyadenine and 8-hydroxyguanine have been found as products of the radiolysis of nucleosides and DNA. We have prepared the nucleoside derivatives of these compounds for conjugation with BSA. For the synthesis of 8-hydroxyadenosine, 8-bromoadenosine, which is commercially available, was refluxed in acetic anhydride in the presence of sodium acetate. The resulting N-acetyl-8-hydroxyadenosine was incubated in concentrated ammonia at room temperature to remove the acetyl group. After removing acetamide by chloroform extraction, 8-hydroxyadenosine was recrystallized from water. 8-Hydroxyguanosine was prepared by essentially the same procedure as 8-hydroxyadenosine except that 8-bromoguanosine was synthesized by bromination of guanosine.

To produce the immunogens, 8-hydroxyadenosine and 8-hydroxyguanosine were conjugated to BSA and RSA by the method of Erlanger and Beiser. This involves the periodate cleavage of the ribose ring, conjugation to free amino groups in the albumin, reduction of the Schiff base and stabilization of the conjugate by sodium borohydride. The number of haptenic groups was determined by UV absorbance. Rabbits were immunized with the BSA conjugates and polyclonal antibodies were produced.

The antibody to 8-hydroxyadenine has a very high titer, giving a strong signal with the RSA conjugate at a 1:10,000 dilution. The IC_{50} for 8-hydroxyadenosine was 20 M. No inhibition was observed with 8-bromadenosine, adenosine, guanosine or inosine at levels of 1.25 mM. Polyclonal antibodies have also been obtained with the 8-hydroxyguanosine conjugate. Results of this work are in preparation for RADIATION RESEARCH.

PLANS FOR THE UPCOMING PROJECT PERIOD

We plan to continue with our future plans as originally proposed. We will work on optimizing our present assays to their limits of detection. We also plan to prepare antibodies to 5-hydroxy-5-methylhydantoin, formamidopyrimidine and 5-hydroxyuracil, major DNA radiolysis products.

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