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REPAIR OF DNA TREATED WITH γ-IRRADIATION AND CHEMICAL CARCINOGENS

Progress Report

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PROGRESS REPORT

1. 3-methyladenine Glycosylase in Rat Liver Nuclei

Work on this enzyme has been completed for the present by G.R. Cathcart who has written his Ph.D. thesis and has submitted a paper to the Journal of Biological Chemistry. A copy of the manuscript is included and an abstract is as follows:

A 3-methyladenine-DNA N-glycosylase was identified in both nuclear and cytoplasmic extracts of rat liver. The enzyme was purified from nuclei due to a lower level of non-specific nucleases. The enzyme was purified 100-fold using DNA-cellulose and phosphocellulose chromatography. Several methods of assay were developed and are discussed.

The molecular weight of the glycosylase was 24,000. It exhibited a preference for double-stranded alkylated DNA. The apparent K_m for 3-methyladenine residues was 2.6 nM. The enzyme was stimulated maximally by the addition of 40 mM NaCl. The addition of 1 mM EDTA had no effect on activity, but Mg^{++} did stimulate the base release. The enzyme was shown to be a glycosylase by the stoichiometric release of 3-methyladenine, and the appearance of alkali sensitive sites. There was no endonucleolytic activity on double-stranded DNA, depurinated DNA, UV treated DNA or γ-irradiated DNA. The phosphocellulose fraction was contaminated with an endonuclease activity specific for single-stranded DNA which could be removed by chromatography on Sephadex G-100.

The enzyme preparation also released 7-methylguanine and the rate of release was approximately 15% of that of 3-methyladenine. The relative rate of release of 3-methyladenine and 7-methylguanine were the same for both the DNA cellulose and phosphocellulose fractions and both activities were inactivated by heat to the same extent. Whether one or two enzymes are involved is not known. The preparation does not release 3-methylguanine or 1-methyladenine.

2. Characterization of a 7-methyguanine N-glycosylase activity in E. coli

During the purification of the 3-methyladenine activity in E. coli using DNA reacted with [^{3}H]MNU it was observed by HPLC analysis that there was some release of 7-methylguanine. Under some conditions this release was negligible and under other conditions the release amounted to a fair percentage of the 3-methyladenine released. The ratio of 7-methylguanine to 3-methyladenine varied inversely with the amount of substrate and directly with the amount of enzyme. This data is difficult to explain either by a single or by two separate enzymes. Various other experiments have been done to try to clarify this problem. One approach has been to make a substrate with a negligible amount of 3-methyladenine in it. By testing varying pH's, temperatures and times of incubation, it was possible to produce a substrate 3-methyladenine to 7-methylguanine ratio of 0.0074 compared to the original DNA with a ratio of 0.12. The amount of single stranded DNA in this preparation was checked by hydroxyapatite and found to be only 1-3%. Analysis on CsCl also confirmed the double stranded nature of the substrate. Although divalent metal is not required for the reaction with 7-methylguanine, it stimulates the reaction. A pH optimum of 8.0 for the reaction has been determined and an apparent K_m for the 7-methylguanine of 2.8×10^{-9} M has been established.

The current problem is to determine whether this 7-methylguanine activity is due to the same enzyme which catalyzes the release of 3-methyladenine or to a different enzyme. This will be approached by further purification and assay with

both substrates, by the use of inhibitors to measure Ki values, and by heat inactivation studies. Since a number of important carcinogens such as aflatoxin and chemotherapeutic agents such as BCNU react with the 7 nitrogen of guanine, these will be tested with this enzyme preparation.

3. [^{3}H]MNU treated DNA in nucleosomes as a substrate for 3-methyladenine N-glycosylase

Experiments in this laboratory are underway to define the susceptibility of the alkylated DNA in various chromatin structures to 3-methyladenine N-glycosylase. We are using the E. coli enzyme as the probe. Several preparations of nucleosomes from chicken erythrocytes have been made using both an old and a new procedure of Dr. Don Olins. These nucleosomes have been characterized both as to the size of the DNA (140 base pairs) and the proteins which are present. The alkylation of the DNA of these nucleosomes has been compared to that of free DNA, and under similar conditions, it has been observed that there is approximately 50% alkylation of nucleosomal DNA compared to free DNA. This shielding effect of histones does not appear to be primarily in the major or minor groove of the DNA as the ratios of 3-methyladenine to 7-methylguanine and 0-6-methylguanine are not significantly different from the ratios of free DNA.

Preliminary experiments with alkylated nucleosomes showed that the 3-methyladenine N-glycosylase released 3-methyladenine and this reached a plateau of approximately 28% of the total. Re-examination of these nucleosomes by sucrose gradient and analytical ultracentrifuge revealed a 6.7 rather than an 11 s_{20w}. The reason for this is not clear but a new preparation is being made by a different method and these nucleosomes will be retested. Then we will try to see if they can be altered by such things as ionic strength, chemical or enzymatic acetylation, ethidium bromide, and chromatin proteins, to release more 3-methyladenine.

4. Isolation and Characterization of an enzyme from E. coli and mammalian tissue active on 2,6-diamino-4-hydroxy-5-N-methyldormamido-pyrimidine in DNA

An N-glycosylase active on this derivative of 7-methylguanine, in which the imidazole ring has been opened, has been described in E. coli by Chetsanga and Lindahl. We have made this substrate, examined its altered base on HPLC and have found in a preliminary screening of E. coli fractions, this activity. We propose to determine whether this enzyme recognizes any similar type of lesion in γ-irradiated DNA, and if so to try to characterize this lesion.

5. Correlation of x-ray sensitivity with removal of alkylated bases from DNA in x-ray sensitive and x-ray resistent lines of lymphoma cells

We have started a collaborative study with Dr. Tom Yau in the Department of Radiobiology. He has isolated a series of cell lines from the parent murine lymphoma L-5178y which are either more radiosensitive or more radioresistant. We have worked out most of the technology to examine the loss of bases alkylated with [^{3}H] methylnitrosourea. In preliminary in vivo studies with the non-mutant strain there is a restricted loss of 0-6-methylguanine, a greater loss of 7-methylguanine and a rapid loss of 3-methyladenine with a half life of about four hours. The experiments need to be refined and then we shall examine the various strains to look for variations in removal. Besides this approach, we plan to look for induction of repair MNU damage with this system. We also wish to try to develop resistant strains similar to those of the dihydrofolate reductase system.

6. The reaction of β-Propiolactone with Derivatives of Adenine and with DNA

A manuscript with this title has been prepared and will be submitted to Biochemistry. An abstract is as follows:

The reaction of deoxyadenosine with β-propiolactone produces two derivatives. The first is 1-(2-carboxyethyl)β-D-deoxyribosyl adenine (CEdA) first described by Maté, Solomon and Segal (1977). The proposed structure for the second is 3-(β-D-deoxyribosyl)-7,8,dihydropyrimido[2,1-i]purine-9-one (dDPP). Spectral characteristics of both compounds are presented. These include UV spectra at acid neutral and alkaline pH's, infrared spectra, fluorescence spectra, and NMR spectra. The extinction coefficient for CEdA is 12,900 at 258 nm and that for dDPP is 12,400 at 305 nm. The dDPP was converted to CEdA by mild acid hydrolysis, and the CEdA was converted to dDPP by reaction with a carbodiimide derivative.

When poly A was reacted with β-propiolactone, the yield of DPP in the polymer was 7.9%. When DNA was alkylated by [^{3}H]β-propiolactone at relatively high concentrations and then acid hydrolyzed to 1-(2-carboxyethyl)adenine (CEA) and 7-(2-carboxyethyl)guanine (CEG), a CEA to CEG ratio of up to 0.62 was obtained. With relatively low concentrations of [^{3}H]β-propiolactone, the ratio with double stranded calf-thymus DNA was 0.05-0.06 while with the same DNA, single stranded it was as high as 0.43. Some stability studies of the β-propiolactone derivatives are presented.

Manuscripts

1. Cathcart, G.R. and Goldthwait, D.A. Enzymatic Excision of 3-Methyladenine and 7-methylguanine by a Rat Liver Nuclear Fraction. Submitted to J. Biol. Chem. and included.

2. Chen, R., Mieyal, J., and Goldthwait, D.A. The Reaction of β-Propiolactone with Derivatives of Adenine and with DNA. Ready to be submitted to Biochemistry.

Effort of Principle Investigator

Twenty percent of the time of the principle investigator is involved with this project.

Preprint Removed

Department of Energy Contract Ey-76-S-02-2725

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Report of Equipment Purchased

Waters Associates -	High Performance Liquid Chromatography Programmer	$2,000
	Injector	$1,300
Gel Electrophoresis Apparatus (being purchased)		