COO-3286-17

STUDY OF THE RADIATION EFFECTS ON NUCLEIC ACIDS AND RELATED COMPOUNDS

Progress Report
for Period August 15, 1976 - November 14, 1977

Shih Yi Wang

The Johns Hopkins University
Baltimore, Maryland 21205

August, 1977

Prepared For
THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
UNDER CONTRACT NO. EY-76-S-02-3286

DISCLAIMER

NOTICE

ABSTRACT

We are investigating the effects of ionizing radiation on nucleic acids and components. Our approach involves four levels of operation and progress is being made at each stage. First, procedures were established to separate and purify three reactive radiation products from thymidine. Second, improved methods of synthesizing trans-glycols of pyrimidines were developed, and a new method for the stereospecific synthesis of cis-glycol of pyrimidines was realized. Thirdly, the Ames Salmonella test was used to determine the mutagenicity of the radiation products and the reactive ones from thymine and thymidine were found to be highly mutagenic. Therefore, all radiation products should be considered potential human health hazards and should be screened when they can be purified and synthesized. In the fourth stage, the reaction of each nucleic-acid base with Cu^{++} and cis-5,6-dihydro-6-hydroperoxy-5-hydroxythymine (6-TOOH) was studied in order to further our understanding of the molecular mechanisms of radiation mutagenesis. The presence of Cu^{++} was shown to be necessary for the effective mutagenic activity of 6-TOOH in the H. influenzae transformation assay. These findings provide fundamental information about the possible health hazards of ionizing radiation and will be useful in designing methods to protect against and repair radiation damage, which may be mutagenic and carcinogenic.

A. SCOPE AND OBJECTIVES

During the past several years, we have been investigating the chemical and biological effects of ionizing radiation of nucleic acids. To study these effects, we have been following a course of action which involves four levels or stages of operation. These stages are independent for each radiation product studied, however, several radiation products can be examined simultaneously. The purpose of these stages are:

1. to establish procedures for the separation, isolation, and characterization of major radiation products of pyrimidines, pyrimidine nucleosides, etc.;

2. to develop methods for the synthesis of these radiation products once they are identified;

3. to examine the apparent biological effects for each radiation product in vitro and in vivo; and

4. to study the molecular mechanism related to an observed radiobiological phenomenon.

A somewhat more detailed discussion of these stages in our research is presented in the accompanying Renewal Proposal.

In this examination of the basic mechanisms of the effects of ionizing radiation on biological systems, our goals are to provide fundamental information about the health hazards caused by ionizing radiation and to design methods for protection against and repair of any such damage. This knowledge will be of value in efforts to preserve and improve the environment and thus promote the health and welfare of man.

B. SIGNIFICANT RESULTS

Members of the Hopkins' community have given us a great deal of support through their collaboration in this work because our results are considered by many to be not only interesting, but also quite significant. Under this

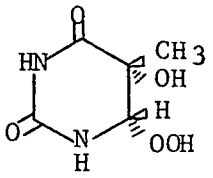
circumstance, substantial advances were made last year, especially in view of the extremely low level of our funding. The following report briefly describes our progress in each stage during the last 12 months.

B.1. Since we were successful in the identification of thymine radiation products, [J. Amer. Chem. Soc., 94, 4764 (1972); Biochem. Biophys. Res. Commun., 54, 1224 (1973); J. Org. Chem., 41, 567 (1976)] a similar approach has been used to separate, isolate, and characterize the radiation products of its nucleoside, thymidine. Thymidine, with its ribose moiety, exhibits a much more complex radiation reaction than thymine. Because of strenuous efforts to separate three reactive radiation products from among the more than twenty products that are formed, we are now able to obtain these three products in their pure states. Although the structures of these three have not been established yet, their biological effects have been tested and the results of those tests are quite interesting (see B.3.). We are presently involved in the process of collecting the pure compounds in sufficient quantities for determining their actual identifications. Dr. Jean Cadet (Centre D'Etudes Nucleaires de Grenoble, Commissariat a L''energie Atomique, France), who has published extensively in this area, was a Visiting Associate in our laboratory for four months and his collaboration in this effort was very helpful. He is planning to visit again for two weeks in September.

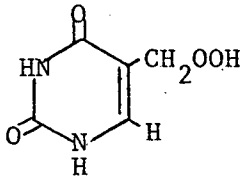
B.2. Because pyrimidine glycols have been identified as radiation products and because they have been used as the starting materials for the preparation of pyrimidine hydroperoxides [several of which have been shown to produce a number of biological effects, see B.3.], we are extremely interested in improving the methods for synthesizing these glycols. We have been quite successful in this endeavor. A report describing our methods appeared in print in Biochemical and Biophysical Research Communications (copies of a reprint are enclosed). In addition, our comparative studies indicate that various isomeric hydroperoxides

may lead to quantitatively and qualitatively different biological effects. Therefore, we are extremely interested in developing stereospecific syntheses of various compounds, especially the starting materials, pyrimidine glycols. Recently, we realized the stereospecific preparations of cis-isomers of pyrimidine glycols. A copy of a manuscript concerning this development is enclosed. [These publications provide molecular structures for these compounds.]

B.3. Our study of cis-5,6-dihydro-6-hydroperoxy-5-hydroxythymine (6-TOOH) as a mediator in ionizing radiation mutagenesis permitted us to propose a molecular mechanism for in vivo radiation mutagenesis. On this basis, we assumed that most compounds containing a hydroperoxy moiety (OOH) were mutagens and that these



6-TOOH



α-TOOH

compounds may be quantitatively different because they have varying chemical reactivity. However, in our study of the mutagenic action of 5-hydroperoxymethyluracil (α-TOOH), we found that α-TOOH is 10^3-fold more effective than 6-TOOH in the inactivation of H. influenzae transforming DNA. This was unexpected because α-TOOH is 10^3-fold less reactive chemically than 6-TOOH. Therefore, it should be much less effective than 6-TOOH if chemical reactivity is the sole basis for biological inactivation.

In order to further our understanding of mechanistic differences in the 6-TOOH and α-TOOH induced mutations, we are searching for factors other than purely chemical effects. Methodology for detection of bacterial mutations developed by Ames et al. [Mutation Res., 31, 347 (1975)] appeared to serve this purpose. In collaboration with Professor Ernest Bueding and R. Batsinger such a study is being carried out. Besides α-TOOH and 6-TOOH, three hydroperoxy derivatives of thymidine, Thd-2, Thd-3, and Thd-4, are also being tested for their mutagenic activities. Furthermore, a thymidine glycol and a thymine

glycol are being used for comparative purposes.

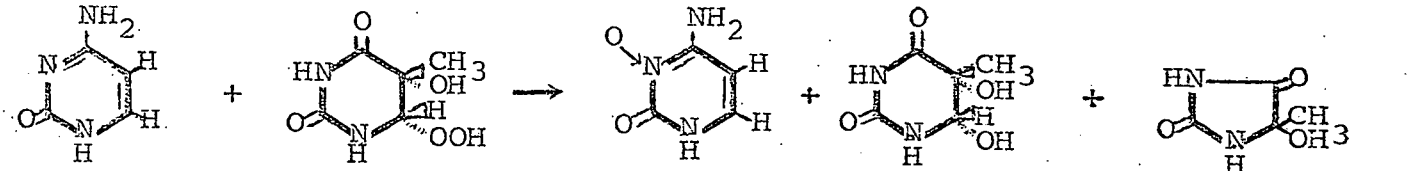
The test procedures of Ames employ histidine requiring mutants of Salmonella typhimurium which have been selected for their sensitivity and specificity. Of the two strains used, TA100 is sensitive to base pair substitutions and TA98 to frameshift mutations. In addition, both strains contain three supplementary features which greatly increase their sensitivity to mutagens: one causes the loss of the excision repair system (Δuvr B), another leads to defects in the lipopolysaccharide cell wall (rfa), and the other results in ampicillin resistance rendered by a resistant transfer factor (R factor).

The results of the test among seven radiation products are listed in Table 1. Five of them had high mutagenic activities while the two glycols exhibited very little mutagenic action. In general, these hydroperoxy derivatives required no metabolic activation (see columns 2 and 5 in Table 1) by the use of a microsomal/cytosol fraction, S_9, from the liver of rats induced either with a polychlorinated biphenyl mixture (Aroclor 1254, columns 3 and 6 in Table 1) or phenobarbital (columns 4 and 7 in Table 1). While little or no activity was observed with strain TA98 in most cases, extremely high mutagenic activities ranging from 3.2 to 40 revertants per nmole was detected with strain TA100 for these mutagens. These results suggest induction of base-substitution or missense rather than frameshift mutations. It should be pointed out that the highest mutagenicity activities of these hydroperoxy compounds was exhibited by Thd-2 with 48 revertants per nmole produced in the presence of Aroclor induced microsomes. Under similar conditions, Thd-3 also exhibited a somewhat higher activity than that without metabolic activation. Also, it should be noted that Thd-4 had high mutagenic activities with strain TA100 in the absence of microsomes and with TA98 in the presence of microsomes induced by phenobarbital. Clearly, these hydroperoxy derivatives are mutagenic in Ames Salmonella test when this is compared with the extremely low (barely significant) activities of Thy glycols.

The toxicity, growth inhibiting activity, of the radiation products for

S. typhirium was found to be of a relatively high order (Table 2) with the exception of the two glycols whose approximate I_{50} values are several logs higher. If this is a reflection of the general toxicity of these compounds in man, radiation products should be considered potential human health hazards and should definitely be screened when they can be purified and synthesized.

B.4. Taking into account that 6-TOOH, as a mutagen, is most effective in the presence of Cu^{++} when assayed by the H. influenzae transformation method and acts only with missense-sensitive strain TA100 of Salmonella typhirium in the Ames test, it may be theorized that this mutagenesis is caused by modified DNA bases which result from the reaction of the base with 6-TOOH and Cu^{++}. If 6-TOOH is produced by radiation in situ, then the neighboring bases are the most logical targets for action. Earlier, we showed that, in the absence of metal ions, 6-TOOH produced changes in guanine, thymine, and cytosine [Radiation Res., 59, 274 (1974)]. Cytosine was converted to N(3)-oxide in the presence of 6-TOOH, which, in turn, reacted to give thymine glycol and 5-methyl-5-hydroxyhydantoin.



Now, we find that in the presence of Cu^{++}, the same reaction occurs. Cu^{++} was found to form a stable complex with 6-TOOH thus increasing the lifetime of the unstable 6-TOOH. This complex renders 6-TOOH more effective as a reagent for the solute rather than decomposing readily with the medium or the solvent. This is reflected as higher mutagenic activity for 6-TOOH. This finding is unexpected because it is generally assumed that hydroperoxides will interact with transition metal ion to form free radicals which then react with and modify other bases of the cell's genome. This antithesis may be a key to the understanding of reaction mutagenesis. Another interesting finding is that cytosine N(3)-oxide tends to

decay to cytosine when standing. If cytosine N(3)-oxide reversion should occur *in vivo*, will it also abolish mutagenic action of cytosine N(3)-oxide on standing? Indeed, this and other findings merit further study so that molecular mechanisms of radiation mutagenesis can be elucidated.

C. EFFORT OF THE PRINCIPAL INVESTIGATOR

The principal investigator has devoted much more time on this project than the 10% indicated in the budget proposal for last year and the low level of funding precluded any compensation for the principal investigator. The accompanying new budget proposal shows a 20% level of effort for the principal investigator with a request for comparable support. However, he will probably attend to this project with a great deal more effort than 8 hours per week.

D. LIST OF PUBLICATIONS

1. B.S. Hahn and S.Y. Wang, "The Preparation of *trans*-Pyrimidine Glycols by Near-UV Irradiation," Biochem. Biophys. Res. Commun., 77, 947 (1977).

2. H.S. Ryang and S.Y. Wang, "α-Diketone Sensitized Photo-oxidation of Pyrimidines," in preparation (manuscript enclosed).

3. J.E.T. Kelley, "The Evaluation of *cis*-5,6-Dihydro-6-hydroperoxy-5-hydroxythymine (6-TOOH) as a Radiomimetic Compound." Ph.D. Thesis, The Johns Hopkins University, June, 1977 [Thesis advisor: Dr. Timothy Merz].

Table 1. Mutagenic activities of seven radiation products of thymine and thymidine on S. typhimurium strains TA 100 and TA 98 in vitro with (+) and without (-) added rat liver microsomal fraction (S_9).

Compound	Number of Revertants per nmole in Excess of Control[a]					
	TA 100			TA 98		
	$-S_9$	$+S_9$ Ar[b]	$+S_9$ Ph[b]	$-S_9$	$+S_9$ Ar[b]	$+S_9$ Ph[b]
α=TOOH	5.2	5.2	4.4	0.8	0.7	1.4
6-TOOH	9.3	2.9	0	1.4	0.8	1.4
Thd-2	15	48	0	0	0	0
Thd-3	3.2	8.8	0	0	0	0
Thd-4	40	0	33	0	0	17.5
Thy glycol	0	0.01	0.04	0	0	0
Thd glycol	0	0.02	0.03	0	0	0.05

[a]Number of spontaneous revertants averaged 165 and 24 for TA 100 and TA 98, respectively.

[b]Assays were carried out in the presence of S_9 fractions from either aroclor (Ar) or phenobarbital (Ph) treated rats.

Table 2. Growth inhibitory effects of seven radiation products on S. typhimurium strain TA 100 on agar plates in the presence of 6 mM histidine.

Compound	Concentration (nM/Plate)	Inhibition %	Approx I_{50} (nM/Plate)
α-TOOH	127	41	
	43	29	150
	13	12	
	4	0	
6-TOOH	113	68	
	34	25	80
	11	15	
	3	0	
Thd-2	110	59	
	36	33	
	7	14	90
	1.5	9	
	0.4	0	
Thd-3	110	66	
	36	20	80
	7	0	
Thd-4	110	67	
	36	12	
	7	11	80
	1.5	5	
	0.4	2	
Thymine glycol	63,000	22	
	6,300	8	200,000
	630	0	
Thymidine glycol	36,700	15	
	3,700	1	150,000
	370	0	