STUDY OF THE EFFECTS OF RADIATION ON
NUCLEIC ACIDS AND RELATED COMPOUNDS

Progress Report

MASTER

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ABSTRACT

Ionizing radiation produces genetic effects in biological systems. Since genetic effects are usually the result of modification of DNA or sometimes, of RNA, a great deal of interest is being centered on the chemical and physical nature of radiation-induced lesions to nucleic acids and their components under various conditions. The results of these investigations have revealed the enormous complexity of chemical events in these systems and the possible degradation of nucleic acids by strand breakage. Therefore, work in the ionizing radiation of DNA and its components has proceeded along a dual course. On the one hand, chemical changes have been characterized on the molecular level for a number of primary radiation products. On the other hand, strand breakage has been investigated intensively as a direct primary event. Both of these aspects were emphasized in our research last year. We succeeded in the synthesis of a nucleoside of TOO_H, i.e., 5-hydroxy-6-hydroperoxy-5,6-dihydrothymidine, a compound more closely resembling the natural products than TOO_H. TOO_H causes chemical modifications of pyrimidine bases and results in changes concomitantly. TOO_H leads to the production of DNA chain breakage or chromosomal or chromatid aberration of cells. Thus, the effects of TOO_H are radiomimetic and are being studied as a possible mediator of radiobiological effects.

Introduction

Ionizing radiation produces genetic effects in biological systems. Since genetic effects are usually the result of modification of DNA or, sometimes, of RNA, a great deal of interest is being centered on the chemical and physical nature of radiation-induced lesions to nucleic acids and their components under various conditions. The results of these investigations have revealed the enormous complexity of chemical events in these systems and the possible degradation of nucleic acids by strand breakage. Therefore, work in the ionizing radiation of DNA and its components has proceeded along a dual course. On the one hand, chemical changes have been characterized on the molecular level for a number of primary radiation products. On the other hand, strand breakage has been investigated intensively as a direct primary event. In view of this, the last renewal proposal has emphasized two aspects of our research. One is the continued effort to isolate pure radiation products. The second is the initial attempt to study the chromosome aberrations caused by a radiation product. We believe that the possible relationship between primary chemical alteration and strand breakage should be investigated. Substantial progress has been made in both areas which are summarized below.

Isolation of Radiation Products

The problem of preparing a sufficient quantity of radiation products is our primary concern at this time. It is generally acknowledged that the most important breakthrough in photochemistry and photobiology of nucleic acids was the identification and characterization of a thymine dimer. This dimer may be obtained in adequate amounts for the study of its role in chemistry and biology. However, in the study of x- or γ -radiation effects in biological systems, chemists have been searching for a similar possibility for the past fifty years. There seems to be no question that ionizing radiation produces peroxides with biomolecules, and yet the investigation of the role of peroxides in γ -radiated biological systems has a long and not particularly fruitful history. Therefore, we were extremely excited about our actual isolation and identification of cis-5-hydroxy-6-hydroperoxy-5,6-dihydrothymine (TOOH), the major product resulting from γ -radiation of aerated aqueous solutions of thymine and, presumably, of DNA. In addition, we are able to synthesize TOOH and to render it "stable". Thus, its interaction with nucleic acid components, prokaryotic and eukaryotic cells, chromosomes, etc., can be studied (see below). We thought that it may not be presumptuous to view what we have

accomplished in TOOH as similar to that in the thymine dimer as expressed in the last progress report. If the role of TOOH does not encompass all of the radiation effects in biological systems, it certainly will produce explicitly the effects of peroxides. It seems appropriate that TOOH should be evaluated as a possible chemical intermediate in radiobiological processes.

In this regard, we put forth two goals. One is to prepare hydroperoxides of thymidine and/or thymidylic acid. These compounds more closely resemble those of natural products and understandably have additional properties desirable for the study of biological processes. The second goal is to explore various reaction conditions in order to simulate γ -radiation reactions. This approach would not only make the study of radiation reactions more manageable but also would facilitate the isolation of radiation products in milligram quantities. Ordinarily, the amount of various radiation products are so minute in one irradiation experiment that the separation and accumulation of sufficient quantities of each is an extremely tedious process. As to the first goal, we succeeded in the synthesis of 5-hydroxy-6-hydroperoxy-5,6-dihydrothymidine, a nucleoside of TOOH. TOOH is a difficult compound to handle; therefore, its nucleoside should be more

complicated to achieve. Attempts are now being made to separate the cis- from the trans-isomer. As to the second goal, photolysis (313 and 360 nm) of pyrimidine derivatives in the presence of hydrogen peroxide (HOOH) was found to produce photoproducts which are identical to γ -radiation products. In particular, thymine, thymidine, uracil, and cytosine have been studied. In principle, HOOH in aqueous solution is decomposed to oxygen (O_2) and water (HOH) by light of wavelengths shorter than 366 nm, and the following reaction scheme has been widely accepted.

Therefore, the oxidative species ($\text{HO}\cdot$, $\text{HOO}\cdot$, and O_2) induced by this photolysis are the same as those induced by radiolysis of aerated aqueous solutions. As estimated by the decrease of optical density readings at absorption maxima, the rate of reaction in decreasing order is Ura > Cyt > Thy > Thd for both 313 and 360 nm light. So far, five, two, six, and four products from Ura, Cyt, Thy, and Thd, respectively, have been identified among numerous end products. These compounds have their counterparts respectively in the radiation products. This approach not

only facilitates our isolation and identification study but also serves as a comparative study of photo- and radiation chemistry of nucleic acids, a study of the effects of near-UV light on biological systems as well as a study of the possible mechanism of aging, etc. This work will be reported in the forthcoming meetings of the International Congress of Radiation Research at Seattle and of the American Society for Photobiology at Vancouver, B.C.

Interaction of TOOH with Pyrimidines and Biological Systems

Hydroperoxides of nucleotides have been shown to be produced in nucleic acids as a result of exposure to ionizing radiation. Consequently, it is tempting to ask, "What is the mode of action of a hydroperoxy moiety existing in a nucleic acid chain or of a hydroperoxy thymine present in a DNA precursor pool of the cell?".

This leads to the following questions.

- 1) What is the result of the action of TOOH on neighboring moieties?
- 2) Does TOOH inactivate cells and transforming DNA and cause mutagenesis?
- 3) Does TOOH lead to the production of chain breakage or chromosomal or chromatid aberration of cells?

Preliminary experiments related to these questions

indicate that TOOH at very low concentrations has radio-mimetic properties. TOOH is not sensitive to catalase as HOOH which was most commonly used for the study of deleterious radiation effects and only at super-biological concentrations. Thus, TOOH seems to be an ideal reagent for these studies which yielded some interesting observations and which are summarized below.

Effect of TOOH on Nucleic Acid Bases

This study was undertaken to evaluate the effect of TOOH, if formed in DNA, on its neighboring bases or vice versa. When Ade, Gua, Cyt, Thy, or Ura was treated with an excess of TOOH, Ade and Ura were found to be resistant to TOOH while Gua, Cyt, and Thy gave rise to many products. There are two interesting findings: 1) Cyt is converted to N(3)-oxide in the presence of TOOH which, in turn, reacts to give 85% 5-methyl-5-hydroxyhydantoin and 11% Thy glycol and 2) Thy in the presence of TOOH yields Thy glycol as the product for both compounds. This demonstrates the

possible effect of radiation products on the neighboring bases and, also dramatically, the effect of neighboring bases on the eventual fate of radiation products in nucleic acids. These chemical changes should result in biological effects. Therefore, both the chemical changes and their possible biological consequences should be further investigated.

Effect of TOOH on Chromosomes of Cells

Our present understanding of the genetic effects of ionizing radiation is largely in terms of the production of strand breaks in the DNA molecule itself. Assay of ionizing radiation damage to cells is generally based on measurement of 1) chromosomal or chromatid aberration production, 2) reproductive death or lethal sectoring, 3) unscheduled DNA synthesis, and 4) increased mutation rates. Mechanisms proposed for the production of these radiation effects start with the strand break as the initial lesion and proceed through various pathways of repair, misrepair, or non-repair. In contrast, UV radiation effects are viewed as being the result of photochemical alteration of the DNA bases, predominantly through the formation of cyclobutane dipyrimidine. Specific information about the chemical structure of the major intermediates [i.e. pyr<>pyr] in UV irradiated biological systems

has enabled investigation and understanding of the kinetics and enzymology of damage and repair and the genetic control of radiosensitivity. Questions on the relationship between primary chemical alteration and strand breakage of the same or opposite strand should be investigated also. From studies of UV-induced chromatid aberrations it seems likely that base change can lead to strand breaks.

In preliminary studies, TOOH effects have been looked at in four systems: 1) human small peripheral lymphocytes in short term culture, 2) CHO K-1 Chinese hamster cells in culture, 3) V-79 Chinese hamster cells in culture, and 4) vicia faba lateral root tip meristematic cells. Vicia faba cells appear to be insensitive to TOOH at concentrations up to 10^{-3} M. Treatment of human lymphocytes produces achromatic gaps and chromatid aberrations as well as a severe mitotic delay. The two Chinese hamster cell lines showed induction of achromatic gaps, aberrations, and decreased colony-forming ability. In all of these experiments, the TOOH was supplied exogenously in concentrations of 10^{-3} to 10^{-5} M for periods of 2.5 to 4 hours. Obviously, such a situation is not entirely analogous to that occurring within a γ - or x-irradiated cell. Nevertheless, there is evidence that hydroperoxy hydrothymine derivatives such as TOOH are formed in situ in the DNA of CHO and M. radiodurans

cells (results from P.A. Cerutti). Also, it seems possible that ho_2hThy are formed in the DNA precursor pools of the cell. Our results clearly indicate that TOOH should be further evaluated as a possible mediator of radiobiological effects.

Effect of TOOH on DNA

In a closely related study, Dr. Roger M. Herriott of this Department showed that TOOH acts as a mutagen when it is allowed to react with transforming DNA. Since this is another strong line of evidence suggesting the possible importance of TOOH in the study of radiobiological processes, part of a meeting abstract is reproduced as follows.

"----- . Freshly grown and washed H. influenzae adjusted to 1×10^9 /ml in buffered salts were logarithmically inactivated by 10 mM thymine peroxide (sic) at 37°C. A three-log decrease took place in 10 minutes. The proportion of mutant cells resistant to streptomycin or viomycin increased linearly during exposure, reaching levels 10 fold higher than the initial level. Purified transforming DNA carrying the streptomycin resistance marker is also inactivated by thymine hydroperoxide. Simultaneous production of various mutations will be discussed along with the implications that these results have for an understanding of the delayed action of ionizing radiation on cells."

[H. Thomas and R.M. Herriott, 1973, Mutation Research 21,
240; Abstract 54, 1st. Intern. Conf. on Environ. Mutagens.]

Summary

Many lines of evidence have been obtained on the
radiomimetic action of TOO_H, among them are that:

- 1) TOO_H produces achromatic gaps, chromatid aberrations,
and severe mitotic delays as well as decreased colony
forming ability.
- 2) TOO_H inactivates cells and transforming DNA.
- 3) TOO_H is also mutagenic.
- 4) TOO_H causes base changes.
- 5) TOO_H suffers chemical transformations.

Uniquely, TOO_H (its nucleoside is now available) is
the one and only radiation product which is known to
produce these effects. Therefore, a more expanded
and detailed study of TOO_H and its nucleoside as a
mediator in the development of biological damage
resulting from ionizing radiation seems highly
warrented.

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