Title
SEARCH FOR RADIATION DEAMINATIONS IN DNA

Author
Lemmon, Richard M.

Publication Date
1966-04-01

UCRL-16842

University of California

Ernest O. Lawrence Radiation Laboratory

SEARCH FOR RADIATION DEAMINATIONS IN DNA

Berkeley, California

DISCLAIMER

UCRL-16842

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

SEARCH FOR RADIATION DEAMINATIONS IN DNA

Richard M. Lemmon

April 1966

Search for Radiation Deaminations in DNA

Abstract. Aqueous solutions of DNA-^{14}C were subjected to γ-radiation and the nucleic acid then hydrolyzed to the nucleotide level. A specific search was made for the 5'-dCMP to 5'-dUMP conversion in the intact nucleic acid, but no evidence for such a deamination was found. These results tend to minimize a role for nucleic acid deaminations in radiation genetics.

It has been demonstrated that the nucleic acid bases, adenine and cytosine, are deaminated in dilute aqueous solution by ionizing radiation (1,2). Neglecting ring destruction reactions, the deaminated analogs (hypoxanthine and uracil) are among the major products of the radiolyses of these bases. For example, when a dilute (0.1%) cytosine solution is given a γ-ray dose of 2×10^6 rads, it is found that for every 100 molecules of cytosine that disappear, 36 molecules of uracil are formed (2). These observations appear important to radiobiology as they suggest a mechanism for radiation-induced mutagenesis. It is known that the dilute nitrous acid treatment of the amino bases, adenine, guanine, or cytosine, leads to the production of the corresponding hydroxyl compounds, hypoxanthine, xanthine, and uracil, respectively (3,4). Gierer and Mundry have shown that nitrous acid treatment of intact tobacco mosaic virus actually results in the formation of mutants (5). In addition, the alteration of the amino acid sequence in the protein of the mutant virus has been demonstrated by Tsugita and Fraenkel-Conrat (6). It would appear that radiation-induced deaminations ought to play the same role as nitrous acid-induced deaminations.

We decided to search for evidence of radiation-induced deamination in water solutions of intact DNA. To make the search as sensitive as possible, our work was carried on on DNA labeled with ^{14}C (from E. coli

grown on ^{14}C-labeled algae). We sought evidence of the appearance of the specific deaminated nucleotide, 5'-deoxyuridylic acid (5'-dUMP), from 5'-deoxycytidylic acid (5'-dCMP), because the 5'-dUMP is available commercially for use as a carrier in chromatographic work. The other possible deaminated nucleotides from DNA, deoxyinosinic acid and deoxyriboxanthine phosphate, are not commercially supplied. In addition, as noted above, cytosine gives a very high radiation yield of its deaminated analog, uracil. It therefore appeared that our chances of finding deamination might be greatest in the deoxycytidylic acid-to-deoxyuridylic acid transformation.

The DNA-^{14}C was prepared (7) by (a) exposing the algae Chlorella to $^{14}CO_2$, (b) hydrolyzing the algae (2 cc of wet packed cells containing 75 mc) by heating 16 hrs at 100° with 200 cc of conc. HCl in an evacuated, sealed tube, (c) inoculating the hydrolysate (after removing the HCl, and sterilizing) with an E. coli culture, and (d) obtaining the labeled DNA from the bacteria by the method of Kirby (8). We obtained for this work 1.98 mg of DNA-^{14}C with a specific activity of 14.2 μc/mg.

Hydrolyses of the labeled DNA, both before and after irradiation, were performed as follows: The DNA (in aqueous solution) was first hydrolyzed (15 min) to short-chained polynucleotides by bovine pancreas deoxyribonuclease (Worthington Biochemical Corp., Freehold, N. J.) following the method of Kunitz (9). Venom phosphodiesterase (Worthington) was then added to complete the hydrolysis (2 hrs) to the 5'-mononucleotides, following the method of Koerner and Sinsheimer (10). Paper chromatography on the hydrolysate was done on Wahtman No. 1 paper using the solvent systems, (1) n-butanol-acetic acid-water (2:1:1, v/v/v) and

(2) isopropanol-water-12 N HCl (65.6:18.4:16.6, v/v/v). Positions of radioactive spots on chromatograms were determined by autoradiography (Kodak "Blue Sensitive" Medical X-ray Film), and positions of UV-absorbing compounds (which includes all the nucleotides, nucleosides, and free bases) were found by placing the paper chromatogram over a UV lamp ("Mineralight", Ultra-Violet Products, San Gabriel, Calif.) in a darkroom. Amounts of radioactivity in a chromatographic spot were determined with a G-M counter.

All irradiations were done on 38 µg samples (containing 0.5 µc) of the DNA-^{14}C dissolved in 50 µl of water. The samples were frozen into small Pyrex tubes, which were then evacuated and sealed. In order to more nearly approximate in vivo conditions, dissolved air was not removed before the tubes were sealed. The irradiations were carried out in the 10 Kcurie Co^{60} γ-ray source at the Department of Radiochemistry.

Controls on the DNA-^{14}C preparation were made as follows:

(1) A sample of the unirradiated and unhydrolyzed material was chromatographed, along with 75 µg of 5'-dUMP, using the above two solvent systems. About 50% of the material remained at the chromatographic origin (as DNA should), about 25% was smeared out along the isopropanol-HCl direction, and another 25% appeared as distinct spots at various locations on the chromatograms. However, no trace of radioactivity was detectable in the carrier deoxyuridylic spot.

(2) A sample of the unirradiated DNA-^{14}C was hydrolyzed by the deoxyribonuclease-venom phosphodiesterase method. Chromatography of this material (along with carrier, unlabeled 5'-dUMP) showed that only a trace (about 1%) of radioactivity was left at the origin. About 50%

of the total activity appeared in four major spots that were identified as the four nucleotides (5'-dAMP, 5'-dTMP, 5'-dGMP, and 5'-dCMP). The remaining radioactive spots on the chromatograms were not identified. Again, no radioactivity was detectable in the carrier 5'-dUMP.

(3) Two DNA-^{14}C samples were irradiated at 10^6 rads, and two at 2×10^6 rads, to serve as the "irradiated-but-not-hydrolyzed" controls. Chromatography of the resultant material gave, on all four samples, chromatograms very similar to those of the unirradiated and unhydrolyzed DNA-^{14}C. Again, no radioactivity was detected in the spot of carrier 5'-dUMP that was added after the irradiations.

Two samples each of the DNA-^{14}C were irradiated at 1, 2, and 3×10^6 rads (delivered over 10-30 hrs). These doses were chosen because the maximum deamination of free cytosine was observed in this range (11). After the irradiations the samples were subjected to the hydrolysis and chromatography described above. All of the resultant autoradiographs were more complicated, by a profusion of spots and by a less-effective DNA hydrolysis, than were the hydrolyzed controls. (The less efficient hydrolyses of the irradiated DNA is in accord with a similar observation of Shrago (12).) However, in none of the six chromatograms was there detectable radioactivity in the added 5'-dUMP carrier. The carrier spots were counted under conditions where we could detect a conversion of 0.1% of the DNA 5'-dCMP into 5'-dUMP. This sets an upper limit to the amount of deamination of cytosine in intact DNA that could be taking place under the conditions of our experiments. It is probable, therefore, that nucleic acid deaminations do not play the role in radiation

genetics that was suggested by earlier observations of the radiation deaminations of the free bases.

RICHARD M. LEMMON*

Department of Radiochemistry
University of Helsinki
Helsinki, Finland

References and Notes

1. C. Ponnamperuma, R. M. Lemmon, E. L. Bennett, and M. Calvin, Science 134, 113 (1961).
2. C. A. Ponnamperuma, R. M. Lemmon, and M. Calvin, ibid. 137, 605 (1962).
3. J. M. Gulland and E. R. Holiday, J. Chem. Soc. 765 (1936).
4. J. M. Gulland and T. F. Macrae, ibid. 662 (1933).
5. A. Gierer and K. W. Mundry, Nature 182, 1457 (1958).
6. A. Tsugita and H. Fraenkel-Conrat, Proc. Natl. Acad. Sci., U.S., 46, 636 (1960).
7. I wish to thank Mrs. Margaret A. Smith, Lawrence Radiation Laboratory, University of California, Berkeley, for the preparation of the DNA-^{14}C.
8. K. S. Kirby, Progress in Nucleic Acid Research, Vol. 3, (Academic Press, New York, 1964), p. 5.
9. M. Kunitz, J. Gen. Physiol. 33, 349, 363 (1950).
10. J. F. Koerner and R. L. Sinsheimer, J. Biol. Chem. 228, 1049 (1957).
11. C. A. Ponnamperuma, R. M. Lemmon, and M. Calvin, Science 137, 605 (1962).
12. S. Shrage and R. M. Lemmon, University of California (Berkeley) Radiation Laboratory Report, UCRL-11046, October 1963, p. 216.

13. This work was supported, in part, by the U. S. Atomic Energy Commission. It was performed during the tenure (1965) of a Guggenheim Fellowship, for which I am indebted to the John Simon Guggenheim Memorial Foundation. I am also grateful to Professor Jorma K. Miettinen and the staff of the Department of Radiochemistry, University of Helsinki, for many courtesies and aids during the course of this research.

*Present address: Laboratory of Chemical Biodynamics, Lawrence Radiation Laboratory, University of California, Berkeley, California 94720.