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LET Dependence of DNA-Protein Cross-Links

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INTRODUCTION

Ionizing radiation produces a diverse assortment of molecular lesions in cells. Although DNA is not the only target for radiation damage in the cell, it has been one of the most extensively studied. It has been estimated that 1.0 Gray of x rays yields in normal, mammalian cells approximately: 3-6 chromatin breaks, 20-40 DNA double-strand breaks, 150 DNA-protein cross-links (DPCs), 500-1000 DNA single-strand breaks, and 1000 sites of base damage. There is much less information available comparing each of these types of specific DNA lesions following exposure of mammalian cells to ionizing radiations of different qualities. DNA single- and double-strand breaks have been investigated as a function of ionization density, but there is a dearth of data on high-LET (linear energy transfer)-produced base damage and DPCs. This paper briefly recaps characteristics of low-LET radiation-induced DPCs, reviews the little information known on the LET-dependence of DPC induction, and summarizes some preliminary data obtained in our laboratory using charged particle beams with LET values ranging from 32 keV/μm to 183 keV/μm.

LOW-LET RADIATION-INDUCTION OF DPCs

DPC have been measured by a number of techniques including alkaline elution, sepharose chromatography, extraction with organic solvents or with detergents and salt followed by analysis with gas chromatography combined with mass spectroscopy, and nitrocellulose filter-binding. Several important conclusions can be drawn from the work with low-LET radiation: a) Unirradiated cells contain a low level of DPCs and 1-2% of the DNA in these cross-links is tightly associated with a subset of proteins of the nuclear matrix, and preferentially involves DNA regions containing actively transcribing sequences, (1); b) Techniques to measure DPC are not sensitive to low doses of radiation, but within the dose range of 10 to 100 Gy, the amount of DNA which is cross-linked to protein and therefore retained on the filters is an approximately linear function of the total dose (2), c) Ionizing radiation (IR)-induced hydroxyl radicals can cause DPCs (3), d) The mechanism for the IR-induced DPCs is unknown, but evidence exists from studies with protease treatments that the lesions are covalent adducts of protein to DNA, and studies with human cellular chromatin indicate the prevalence of a thymine-tyrosine cross-link after gamma radiation (4, 5), e) Hypoxia enhances IR-induced DPCs (6), and they can be detected by alkaline elution when doses greater than about 10 Gy are given to aerated cells and repair of SSBs is allowed prior to analysis (7), f) DPCs preferentially induced in the DNA of cells irradiated with low-LET radiation under hypoxic conditions may contribute to cell killing when normal DNA-repair mechanisms are compromised (8), and g) More recent DPC studies have extended our understanding of protein involvement in the DPC-induced by low LET-ionizing radiation (9-14).
HIGH-LET RADIATION-INDUCTION OF DPCs

The published work on high-LET radiation induced DPCs is intriguing, but scanty. Hawkins (15) described his measurement of plaque survival, double strand-breaks and DPC yields with phage T7 suspended in tryptone broth and exposed to gamma radiation alone, or to a mixture of fast neutrons and gamma radiation. His data with neutral sucrose density sedimentation patterns indicate that high-density energy deposition in the mixed neutron field enhances the efficiency of DPC production. Eguchi et al. (16) using alkaline elution demonstrated that the fraction of residual DNA lesions remaining in human melanoma cells 6 hours after irradiation with nitrogen ions having an LET of 530 keV/\(\mu\)m was higher than that observed after x-rays, and unlike the case for x-rays, DPCs were included in the residual DNA lesions measured. Jenner et al. (17,18) using the filter-binding assay have found that there are similar yields for the induction of DPC in hamster cells from exposure to ultra-soft aluminum K-shell x-rays, and high-LET alpha particles and low-LET cobalt-gamma rays under hypoxia, but that the induction decreased in the presence of oxygen, especially for the high-LET radiations. They demonstrated an apparent lack of correlation of DPC-induction with cell killing.

We are investigating the dose- and LET-dependence of cellular survival and the yield of DPCs under oxic conditions in a hamster cell defective in nucleotide excision repair and sensitive to DNA cross-linking and its wild-type parent line. This paper reports only the initial particle-induced yields in the normal cells.

METHODS

Cell Lines and Culture Conditions Three different Chinese hamster ovary (CHO) cells, designated CHO-SC1, CHO-Clone 9 and CHO 43.3B were used in these experiments. The SCI and Clone 9 cell lines are normal, and the 43.3B line is a nucleotide excision repair defective mutant of the Clone 9 that is hypersensitive to MMC (mitomycin C) and to UV light (19). All cells were grown in McCoys 5A medium with 15% fetal calf serum. Cultures were in exponential growth at the time of irradiation and prelabeled for 16 hrs with \(3^H\)TdR. Immediately prior to irradiation the labeled media was removed and replaced with non-labeled medium. Cells were irradiated on ice.

Radiation Sources and Dosimetry Charged particle irradiations were completed in track segment exposures at the Lawrence Berkeley Bevalac and 88" accelerators. Ions, residual energies and LET values studied included: 370 MeV/amu neon ions (32 keV/\(\mu\)m), 57 MeV/amu neon ions (100 keV/\(\mu\)m), 20 MeV/amu nitrogen ions (120 keV/\(\mu\)m), and 31 MeV/amu neon ion (183 keV/\(\mu\)m). The exposure techniques and dosimetry have been previously described (20).

DNA-Protein Filter-Binding Assay The modified nitrocellulose filter-binding protocol of Chiu et al., (4) was used for measuring DPCs. The radioactivity retained on the filter was determined by liquid scintillation spectrometry, and the data were used to calculate the percentage of input \(3^H\)DNA bound to the filter. This technique measures the percentage of DNA associated with protein, and not the number of DPC lesions.

RESULTS AND DISCUSSION

Figure 1A summarizes the dose-dependent yield of initial DPCs for the particle beams studied. The cobalt-60 gamma ray DPC yield from
Ramakrishnan et al. (2) are also plotted showing a linear dependence on dose up to near 100 Gy. The high-energy neon-ion DPC yield at 32 keV/μm is similar to the gamma-ray data. The yields for the other high-energy neon-ions at 100 and 183 keV/μm show similar yields at low dose, but saturation at higher doses. The low-energy nitrogen-ion DPC yield shows a distinctly different yield with a non-linear low-dose dependence, followed by linear dependence at higher doses.

Figure 1B presents a conversion of the particle-induced DPC data in Fig. 1A to particle fluence (F) using the relationship: F (particles/cm²) = (6.242 x 10⁶) x Dose (cGy)/LET (keV/μm). Although the data are preliminary, an LET-dependent yield pattern is evident with increases in the percent DPCs appearing with fewer particle fluences at higher LET values. The results however indicate saturation of the DPC yield at high fluences of the 183 keV/μm neon ions. An enhanced yield is observed after exposure to nitrogen ions at 120 keV/μm without any evidence of saturation. These data may indicate a dependence on particle track structure since the high-energy neon-ions at 100 keV/μm are not at a very different dose-averaged LET, but the dimensions of the microdosimetric ionization structure of the tracks for particles of these two residual energies will be significantly different (21).

Figure 1. Yield of particle induced DNA-protein cross-links (A) as a function of dose (Gy), or (B) as a function of particle fluence. Two CHO normal cell lines were used for these studies: Cl 9 or Sc1.

SUMMARY

We have preliminary data indicating a fluence-dependent yield of particle-induced DPC's with a dependency on LET and particle residual energy. Our data indicate that the DPC yield for hamster fibroblasts in vitro irradiated at 32 keV/μm is similar to that reported for hamster cells irradiated with cobalt-60 gamma rays. At 100-120 keV/μm there is some evidence for an enhanced DPC yield with increasing particle fluence, but there are differences in the yields that are dependent on particle track structure.
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