RADIATION BIOLOGY

THE EFFECT OF PROTON RADIATION DAMAGE TO THE RNA ENZYME, RNASE-P

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Exposure to cosmic radiation causes two poorly understood phenomena: cell death and reduced life span. A percentage of cells are killed by deposition of radiation energy directly within the cell nucleus. This effect is predominant at high dose rates for low linear energy transfer (LET) radiation, but varies for heavy ions with dependencies on LET, particle fluence and Z. The remainder of cell deaths are caused by radiation damage to membranes, proteins and other macromolecular structures. Reduced life expectancy may be the result of radiation induced stem cell death or mutation/carcinogenesis. Cell death due to extranuclear damage caused by proton radiation and radiation-induced RNA damage have not been well defined.

The importance of RNA to the living cell was only recently disclosed when it was discovered that RNA molecules were capable of self-splicing activity. The number and complexity of identified RNA enzymes, called ribozymes, has continued to increase, and the number of their known activities has much expanded. These macromolecules are therefore critical to cell function and survival. RNA exists in complex three dimensional structures composed of single-stranded regions, double-stranded regions and triple helices. The structural energies and helix dimensions, however, are unique from DNA and are expected to respond to radiation differently from DNA. The ribozyme RNase-P processes precursor tRNA to its active form by removing a small fragment of RNA sequence required for proper folding of the tRNA molecule.[1] This function provides a relatively simple means for assessing radiation damage.

This preliminary study has examined the effect of high doses of protons delivered to RNase-P molecules *in vitro* at a high dose rate. Small volumes (100 μl) of concentrated RNase-P (200 nM) were exposed to increasing doses of proton radiation from 0 to 1000 krads. The small volume microfuge tubes (0.5 ml) containing the samples were completely filled with mineral oil to prevent phase changes that might result in proton scattering edge effects. The small volume tubes were emersed in ice water contained within

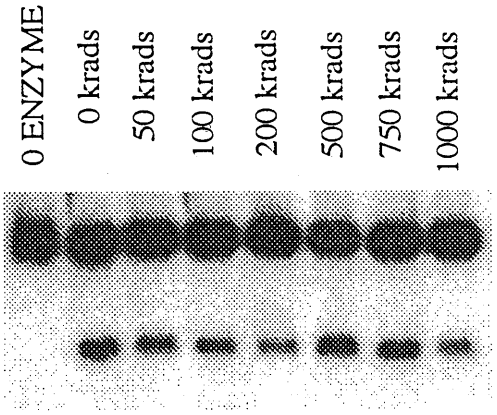

Figure 1. Enzyme activity of proton irradiated RNase-P. Radiolabeled precursor tRNA was incubated with RNase-P that had been exposed to proton radiation (doses noted at the top of each lane). The reaction mixture was run out on 8% urea acrylamide gels, dried and exposed to x-ray film. The top band in the gels indicates the amount of precursor tRNA, the lower band indicates the amount of the smaller tRNA product. The amount of product indicates the activity of the ribozyme.

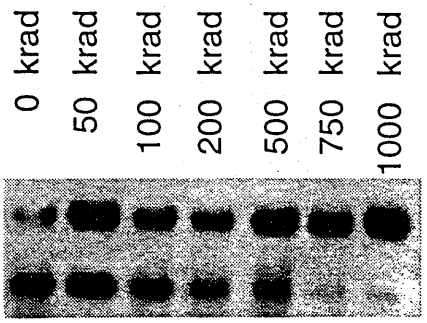

Figure 2. Loss of enzyme activity following ribozyme denaturation. Samples identical to those of Fig. 1 were heated at 65 °C for 5 min and allowed to renature at 37 °C prior to incubation with the precursor tRNA. Loss of activity was evidenced by the reduction in product radioactivity at 750 krad and above.

15 ml conical plastic tubes and fixed in place. The conical tubes were in turn embedded in an ice slush, and the temperature was thus maintained at 0°C throughout the radiation exposure. Following exposure, the samples were incubated with ^{32}P-labeled precursor tRNA under conditions permissive for ribozyme activity.[1] No reduction in enzyme activity was detected at any of the doses delivered to the RNase-P (Fig. 1).

It was possible that the structure of the RNase-P molecule was sufficient to retain enzyme activity despite numerous strand breaks, or that retention of only the active site and selected tRNA binding sites were required to retain enzyme activity. Proton-irradiated RNase-P was therefore denatured by heating at 65 °C for 5 min prior to reannealing at 37 °C, and the enzyme assay was performed again. Because completely denatured RNA would be a single strand, all strand breaks would cause the dissociation of sequences between damaged bases. The assumption that the RNase-P was completely denatured has not been tested. However, further heating resulted in a loss of renaturation that was indicated by complete inactivation of all samples, including the un-irradiated controls. Enzyme activity was sharply diminished for renatured RNase-P samples which had been exposed to 750 krads or more (Fig. 2). To determine the degree of radiation damage to the structure of RNase-P, the enzyme was produced by *in vitro* transcription from a pool of nucleotides including ^{32}P-labeled GTP. The RNase-P was exposed to 1000 krads of proton radiation and the enzyme was size fractionated on a native polyacrylamide gel (Fig. 3). Although no alteration from the un-irradiated samples in gel mobility could be detected

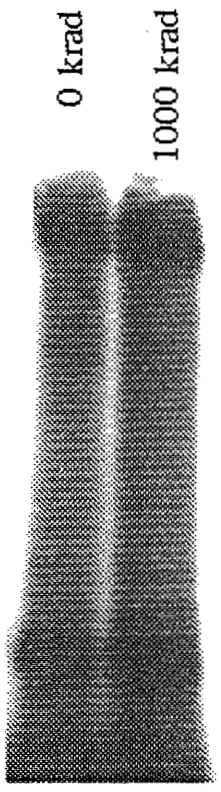

Figure 3. Structural analysis of the irradiated enzyme. Radiolabeled RNase-P was proton irradiated and run out on 8% nondenaturing acrylamide gels. The top band represents intact RNase-P, with three minor structures and one major small product appearing as bands in the lower portion of the gel. Because these bands were present in the un-irradiated control, they were most likely transcription artifacts and do not represent proton damage.

in these samples, the technique is undergoing development and the samples have not yet been analyzed by denaturing urea gels that, like the heat denaturation, should disclose strand breaks not evident in the native enzyme.

1. N.R. Pace and D. Smith, J. Biol. Chem. **265**, 3587 (1990)