UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

MASTER

EFFECT OF DENSELY IONIZING RADIATIONS ON DRY PREPARATIONS
OF LYSOZYME, TRYPSIN, AND DNase

Tor Brustad

August 1958

Printed for the U. S. Atomic Energy Commission

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EFFECT OF DENSELY IONIZING RADIATIONS ON DRY PREPARATIONS

OF LYSOZYME, TRYPSIN, AND DNase

by

Tor Brustad²

Donner Laboratory, University of California, Berkeley, California

The present studies are carried out in order to elucidate the effect on enzyme activity of different kinds of radiation. Beams of protons and alpha particles from the Berkeley 60-inch cyclotron and beams of accelerated nuclei of helium 4, carbon 12, oxygen 16, and neon 20 from the Berkeley Heavy Ion Linear Accelerator (HILAC) have been used. With this variety of particles it has been possible to cover a range of LET 10 times greater than previously utilized in similar studies. The unattenuated energy of the different particles is exactly 10 MeV per nucleon for the HILAC radiation and approximately this value for the cyclotron radiation.

Thin films of enzymes (obtained from Worthington Biochemical Sales Company) were deposited on glass cover slips by pipetting 50 lambda of the enzyme solutions onto each cover slip and then evaporating the solution to dryness in a vacuum desiccator (10^{-3} mm Hg) for 36 hours or more.

The samples were subjected to measured fluxes of the heavy ions in a high vacuum (10^{-5} mm Hg). The experimental apparatus is diagrammed in the first figure.

Figure 1

(As seen from the slide the beam is first deflected by an "analyzer" magnet to insure uniformity in particle momenta. It is then passed through a vacuum tube, 2 meters long, before striking the biological exposure device, constructed as a Faraday cage.)

1. Work supported by the U.S. Atomic Energy Commission.
2. Fellow of the Norwegian Cancer Society, Oslo, Norway. Appointment supported by the International Cooperation Administration under a program administered by the National Academy of Sciences.

after bombardment the samples were recovered into solution and tested for remaining activity against substrate.

The DNase activity was determined by the rate of increase in optical density at 260 m μ when mixed with a DNA solution (Kunitz). The lysozyme activity was determined spectrophotometrically by the rate of increase in optical density at 450 m μ , using a synthetic lysozyme substrate made by Difco Laboratories. The trypsin activity was determined spectrophotometrically by the rate of increase in optical density of hydrolysed benzoyl-L-arginine ethyl ester at 253 m μ (Schwert and Takenaka).

(I: The activity of the enzymes is in all three cases determined by standard spectrophotometric methods)

Figure 2 shows, as an example, the per cent remaining enzymatic activity of trypsin as a function of the dose, the latter expressed as number of heavy particles per cm². Results are given for beams of neon, oxygen and carbon.

In Figure 3 are the results using alpha particles and protons, with the carbon particles as a common reference. Similar results are obtained for lysozyme and DNase.

Figure 4 shows the cross section for inactivation of enzymatic activity, based on 37% remaining activity as a function of the LET of particles used. It is rather interesting to note that:

- a) The cross section for inactivation continues to rise up to the highest LET values studied;
- b) The curves are parallel over the entire LET range studied.

DNase and trypsin have been studied earlier by Smith and Pollard respectively, and their results using deuterons, are included in the

Figure. Smith's results fit very nicely in with ours. Pollard's data on trypsin do not give quite the same good agreement.

Based on the assumption that one primary ionization somewhere within the molecule is sufficient to inactivate the enzyme, Smith and Pollard fit their measured points by these curves and conclude that both enzymes are spherical in shape with molecular weight fairly close to those determined by other physico-chemical methods.

Before our data can be compared directly with such theoretical predictions based on target theory considerations, the contribution to the measured biological effect due only to delta rays must be subtracted to obtain the effect due only to the track core of the heavy ions.

If one defines a delta ray as an energy transfer exceeding a certain value (E_{min}), say 200 ev, one can calculate the energy per unit track length going to delta rays, using Rutherford's formula:

$$Edx = \frac{2\pi e^4}{m} \frac{z^2}{v^2} N Z \ln (E_{max}/E_{min}) dx$$

The total energy transferred is given by the Bete-Bloch formula:

$$dE = - \frac{4\pi e^4}{m} \frac{z^2}{v^2} \ln (E_{max}/I - \beta^2) dx$$

The fraction of the total energy dissipation which goes to delta rays is thus 40% with the threshold set at 200 ev, and 46% with the threshold set at 100 ev.

Applying radiations of 10 MeV/nucleon, relatively narrow LET spectra are obtained when very thin samples are bombarded. Because the different kinds of particles used have the same velocity, their delta ray spectra are identical. It can also be shown that the LET spectrum of a 10 MeV per nucleon alpha particle falls within its own delta ray

spectrum. One can therefore accept that the action of this radiation is very similar to that of the delta rays alone. To the first approximation the delta ray correction can, therefore, be calculated as follows:

One plots the reciprocal of the dose which gives 37% remaining enzymatic activity as a function of the LET of the particles used and subtracts from each of these values 40% of that effect obtained using 10 MeV/nucleon alpha particles. From these corrected $1/D_{37}$ values the corrected cross sections are calculated. These are then plotted as a function of the LET of the track core, instead of the total particle LET.

The results of these calculations are shown in the next figures, where a curve is shown for comparison based on target theory considerations and known molecular weight data.

As one can see, in neither of the cases studied do the corrected cross sections coincide with those predicted on the basis of straight target theory. For the highest LET values studied the difference is about a factor of five. Although the delta ray correction is a subject of some complexity, one feels that the discrepancy between target theory predictions and our corrected results is larger than the uncertainties involved in delta ray correction and other experimental errors.

At the highest LET values used, the energy absorbed by a molecule hit by the track core itself is about 30 times more than that necessary for its inactivation. The discrepancy mentioned above may be explained if there exists some kind of energy migration between protein molecules whereby energy sufficient for inactivation is transferred from a hit molecule to a neighboring one.

Fig. 1

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5

Fig. 6.

Fig. 7.