BIOLOGICAL EFFECTS

Baen-625 Advances in Food Engineering
Effects of Irradiation on pathogens

- Direct effect
  - single and double strand break of DNA

- Indirect effect
  - radiolysis of water
  - formation of OH radicals
Macromolecular target

- The principal target for ionizing radiation is DNA.
- Part of the radiation induced damage will be the result of indirect action of the products of water radiolysis; part of it will be the result of direct action on the DNA molecule.
- Although DNA is the principal target, other bioactive molecules in the cell also undergo deactivation as the result of both direct and indirect radiation damage.
- Very large losses in biological activity of most of the molecular species in the cell other than DNA can be sustained without serious functional deficit.
- Enzymes, for example, are continuously synthesized and damaged molecules are replaced.
Indirect Effect

- Exposure of cells to ionizing radiation induces high-energy radiolysis of water molecules into $H^+$ and $OH^-$ radicals, which are themselves chemically reactive.
- These in turn recombine to produce a variety of highly reactive radicals such as superoxide ($HO_2$) and peroxide ($H_2O_2$), which produce oxidative damage within the cell.
- Most important effect in inactivating pathogens
Direct Actions

- In addition to the reactivity of the products of radiolysis of water, energy deposited by electron might be deposited directly in the biological molecule of interest.

- The initial physicochemical reactions take place in the molecules of the important cell constituents such as DNA rather than in water.

- The result would be ionization and/or excitation in atoms of these molecules and radical formation from the important biological molecule.
Direct effect – base damage

Single strand break; Double strand break; Base damage
**E. Coli – single-strand break**

- DNA of *E. coli* consists of $3.5 \times 10^6$ nucleotide base pair
  - Each with an average $M_w$ of 660
  - Total molecular weigh of $2 \times 10^9$
- Assume $G = 1$ (typical for causing a lesion in a single-strand)
- For 1 kGy
- $\sim 200$ ss-break/*E. coli* DNA molecule

\[
N_m = 10^{-7} GM_w D = 10^{-7} (1)(2 \times 10^9)(1) \approx 200
\]
**e. Coli double-strand lesions**

- DNA of *e. coli*
  - Each with an average $M_w$ of 660
  - Total molecular weigh of $2 \times 10^9$
- Assume $G = 0.07$ (typical for causing a lesion in a double-strand)
- For 1 kGy
- $\sim 14$ ds-break/e. coli DNA molecule

\[
N_m = 10^{-7} GM_w D = 10^{-7} (0.07)(2 \times 10^9)(1) \approx 14
\]
Single- and double-strand break

- Single-strand break
  - Not lethal
  - Result in mutation after replication
  - Repaired
  - However, mutations are weakened and susceptible to environmental effects

- Double strand break is lethal
DNA enzymatic repair
Nature of radiation toxicity has been related to DNA as its principal target.

DNA repair and synthesis depends on proteins (enzymes).

Exposing cells to IR generates a range of potentially harmful molecules called reactive oxygen species (ROS).

ROS cause oxidative stress and can kill cells.

Hydroxyl radicals, one of the primary ROS products of irradiated water (the major component of cells), are particularly toxic to DNA, and can generate other ROS, including hydrogen peroxide and superoxide (a simple peroxyl radical).
The bacteria shown here, *Deinococcus radiodurans* -D. rad-, can survive extreme radiation and repair their own DNA in just 48 hours.

This extremely resilient microbe can endure 100 times the IR levels that kill other bacteria and levels 2,000 times higher than the lethal human dose.
Novel model of IR Toxicity

- Shifting the focus of toxicity and resistance away from DNA damage and repair toward a potent form of protein protection.
- *D. radiodurans* relies not on a highly specialized DNA repair machinery, but on a detoxifying mechanism associated with the microbe’s unusual intracellular environment.
- Most organisms contain near-millimolar concentrations of iron – (contribute to the formation of hydroxyl radicals and superoxide radicals)
Novel model of IR Toxicity

- In resistant bacteria, millimolar Mn(II) concentrations appear to protect proteins from oxidative damage by eliminating superoxide and its derivatives.
- This oxidative protection may in turn shield proteins involved in DNA repair, and subsequently allow them to quickly heal DNA lesions, which in sensitive bacteria turn lethal because their repair proteins are ravaged by radiation.
Electron micrograph of a cross-section of a *D. radiodurans* tetracoccus (cluster of four cells).

- Average abundance of manganese (blue, green, and pink) and iron (red) are shown within a single *D. radiodurans* diplococcus

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Dose-response relationships for pathogen inactivation

- Pathogens inactivation is the end effect of irradiation
- Survival of microorganism exposed to different radiation doses