Iodine Incorporated in Cell Constituents during Sensitization to Radiation by Iodoacetic Acid

Abstract. *Iodine atoms are incorporated in bacterial membrane proteins when cells are irradiated in the presence of iodoacetic acid labeled with iodine-131. Such atoms are produced on reaction of iodoacetic acid with the gamma ray–induced hydroxyl radicals in the surrounding medium.*

Iodoacetic acid and its derivatives such as iodoacetate and iodoacetamide have been shown to sensitize microorganisms to the lethal effects of x-rays and gamma rays (*1*). It was recently reported that the species responsible for such sensitization are the free iodine atoms (*2*) formed by reaction of the sensitizers with the hydroxyl radicals and hydrated electrons produced during radiolysis of water (*3*).

In order to obtain further insight into the mechanism of action of iodoacetic acid, and to locate the sites involved in the process of radiosensitization, we have investigated the incorporation of labeled iodine into constituents of bacterial tissue.

Labeled (I^{131}) iodoacetic acid was prepared by simple exchange reaction. *Escherichia coli* B/r (Hill) was grown in nutrient broth for 18 hours at 37°C. The cells were harvested after three washings with sterile 0.1*M* phosphate buffer and resuspended in sterile phosphate buffer. Labeled iodoacetic acid (20 μc) was added to the suspension, and irradiation was carried out in air with 100 krad of Co^{60} gamma rays. After irradiation, samples were washed three times with sterile phosphate buffer to remove extracellular radioisotope. Chemical fractionation was carried out by a modification of reported methods (*4*). Fractionation into cell wall, cell membrane, and cytoplasmic constituents was effected by treatment of irradiated bacterial cells with lysozyme, followed by sonication (*5*).

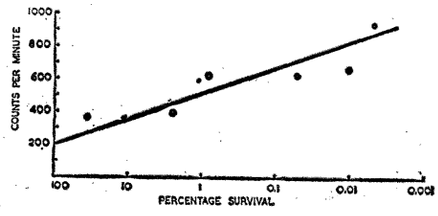

Fig. 1. Survival of cells of *Escherichia coli* relative to incorporation of iodine-131 under an atmosphere of nitrogen.

Table 1. Chemical fractionation of cells. Abbreviations: TCA, trichloroacetic acid; RNAse, ribonuclease; DNAse, deoxyribonuclease; IAA, iodoacetic acid labeled with iodine-131.

Sample irradiated	Insoluble fraction	
	Count per minute	Activity (%)
Treatment with cold 5-percent TCA		
No irradiation (control)	43	100
With IAA added later	17	100
With 0.001*M* KCNS and IAA	46	100
With IAA	35,228	100
Treatment with 75-percent ethanol		
With IAA		97.7
Treatment with RNAse (100 μg/ml)		
With IAA		92.5
Treatment with DNAse (100 μg/ml)		
With IAA		83.0

The chemical fractionation of the irradiated cells has demonstrated that the free iodine is incorporated not in the nucleic acids but in the proteins of the system (Table 1). The presence of a hydroxyl-radical scavenger (KCNS), which is known to reduce the sensitizing effect of iodoacetic acid (*6*), also reduces uptake of the isotope during irradiation; this fact further supports the hypothesis that the sensitization caused by iodoacetic acid is due to the transients produced mainly by the reaction of hydroxyl radicals. Since even after irradiation the bacterial cell wall is impermeable to iodoacetic acid, and radioactivity is not detected in cells treated with irradiated iodoacetic acid (Table 1), these results implicate the release of iodine atoms from the sensitizer in the presence of cells during irradiation. Figure 1 shows that the amount of radioactive material incorporated into the cells is directly related to radiation lethality.

In a radiation-resistant bacterial strain such as *E. coli* B/r there is an enzymic repair system that is postulated to be located in the cell membranes. Since the sensitizing effects of iodoacetamide and iodoacetic acid proved to be more marked in radiation-resistant strains, the inhibition of repair processes after irradiation was believed to be implicated (*7*). On the basis of this hypothesis, the incorporation of radioactive iodine into the proteins of cell membrane would be expected. Our results demonstrate that about 60 percent (36,613 count/min) of the total activity was associated with the cell-membrane fraction; the remainder was more or less equally distributed between the proteins in the cell-wall (12,400 count/min) and cytoplasmic (10,200 count/min) fractions. We cannot say whether these proteins were released from the membrane during the experimental procedure or originally belonged to the fractions concerned.

Thus from these observations one can infer that, during the process of radiosensitization by iodoacetic acid, iodine atoms are incorporated in membrane proteins which may constitute the repair system.

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References and Notes

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Yolk Protein: Structural Changes during Vitellogenesis in the Cockroach Leucophaea maderae

Abstract. *Most of the yolk protein in the mature egg of* Leucophaea maderae *consists of one large component, whereas a second smaller protein is present during the early stages of vitellogenesis. The large protein can be converted to the smaller one and to even smaller units by mild alkaline conditions in vitro. After injection of uniformly labeled leucine-C^{14} into females with developing eggs, the smaller yolk protein becomes labeled first, the label is then transferred to the large protein upon prolonged exposure.*

The most obvious manifestation in the maturation of the oocyte in *Leucophaea maderae* is the deposition of a large amount of yolk. Vitellogenesis begins soon after the imaginal molt and is initiated by secretions from the corpora allata (*1*). During maturation, the protein content of the ovaries increases