Note

Effects of Protein or Polysaccharides on Radiosensitization with NaCl

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It is known that solute in cell suspensions sensitizes or protects the cell from the lethal effect of ionizing radiations. When the cells of *E. coli* capable of repair to DNA damage were irradiated in the presence of alkali-halides, survivals were significantly reduced.^{1~3)} Among the alkali-halides, Cl^- requires relatively higher concentrations to sensitize the cells to radiation than Br^- or I^- . However, the active component for cell inactivation is thought to be X_2^- in each (X; Cl, Br or I) which is produced by the following radiochemical reactions.

Irrespective of such similarity between these halides, some differences can be seen in their radiosensitizing effect on living organisms. Cl^- did not sensitize bacterial spores or fungi to gamma-rays but they were sensitized by Br^- or I^- .^{3,4)} NaCl in a phosphate buffer was equally effective at pH 5 or pH 7 while KBr or KI was less effective at neutral pH than at acidic pH.²⁾ Br^- and I^- sensitized *E. coli* cells more efficiently under anaerobic conditions while no significant oxygen effect was observed in the case of Cl^- .²⁾ These differences have been remained to be explained if the active components for the radiosensitization are analogous to each other such as X_2^- in Eqs. (1) and (2). If the radiosensitization by NaCl is solely due to Cl_2^- , it is hard to explain why the cells were sensitized equally at pH 5 or at pH 7 since the yield of Cl_2^- is much higher at acidic pH than neutral pH.^{5,6)} Furthermore, if Cl_2^- is responsible for the radiosensitization, the recovery of colony-forming units by L-valine is incompatible with its low reaction rate constant.⁸⁾

Of the cells irradiated in the presence of NaCl, recovery from the inhibitory effects on protein, RNA and DNA syntheses were significantly delayed.³⁾ However, no radiosensitization was observed on some mutants of *E. coli* such as *polA* or *recA*.³⁾ From these results it is very plausible that a site(s) where very important cellular functions including repair of DNA damage are located is the target for radiosensitization with Cl^- .

In this paper it will be reported that the radiosensiti-

zation with NaCl is due to the composite effects induced by radiation and changes of ionic strength during the preparation of cell suspensions or dilution of the samples for plating on broth-agar. It will also be reported that the radiosensitization by NaCl can be removed by the addition of macromolecular weight compounds to the irradiation buffer.

E. coli B/r was used throughout these experiments. Cells were harvested at the exponential phase of growth in B₂-broth which contained meat extract (1%), peptone (1%) and NaCl (0.5%). After washing twice with a potassium phosphate buffer (0.067 M, pH 7) the cells were resuspended with the buffer at a concentration less than 10^8 cells/ml. When they were irradiated in the presence of NaCl, equal volumes of the cell suspension and 1 M NaCl dissolved in the buffer were mixed. The cells were irradiated with gamma-rays from ⁶⁰Co under aerobic conditions by air-bubbling during the irradiation. The irradiated cells were spread on the broth-agar to count viable cells after appropriate dilution with the phosphate buffer except where indicated in Table I. Before mixing with cell suspension solutions of the bovine serum albumin, dextran or inulin were dialysed extensively against the phosphate buffer at 4°C to remove low molecular weight impurities which might have been incorporated into the cells during irradiation.

As has been seen in the previous papers,^{3,7)} *E. coli* cells suspended with a phosphate buffer containing NaCl showed lower colony-forming units-ml than cells in the phosphate buffer without gamma-ray irradiation. This effect might be due to changes of ionic strength by the addition of NaCl to the cell suspension and by dilution with the phosphate buffer for plating on broth-agar. If the irradiated cells are more sensitive to the change of ionic strength or if the cells exposed to the change of ionic strength are more sensitive to radiation, the mechanism of radiosensitization by NaCl must be attributed to several factors.

The results in Table I show these composite effects of NaCl. Survival of the cells washed with the phosphate buffer, irradiated in the presence of NaCl and diluted with the buffer were less than 0.01% of those irradiated and diluted with the buffer alone. When the cells irradiated in the presence of NaCl were diluted with a buffer of the same ionic strength, approximately four times higher survivals were observed than the cells treated as described above. A more remarkable effect of the change of NaCl concentration is that the survivals after irradiation with 20 krad of gamma-rays in the presence of NaCl increased 400 fold if there was no change of ionic strength before the cells were spread on broth-agar (Table I, line 4). The cell washed with the buffer containing NaCl were more sensitive to gamma-rays even if they were irradiated in the absence of NaCl (Table I, line 5 and 6). From these results the radiosensitization of *E. coli* by NaCl might be brought about by the composite effects of its presence before, during and after irradiation.

TABLE I. EFFECTS OF NaCl IN THE BUFFERS OF WASHING, IRRADIATION OR DILUTION ON THE COLONY-FORMING ABILITY OF *E. coli* B/r

Washing	Buffer		Colony-forming Units/ml		% Survivals after Irradiation (B/A × 100)
	Irradiation	Dilution	Before Irr. (A)	After Irr. (B)	
PB*	PB	PB	2.0×10^7	5.7×10^6	29
PB	NaCl**	PB	1.2×10^7	3.0×10^2	0.0025
PB	NaCl	NaCl	5.0×10^6	4.8×10^2	0.01
NaCl	NaCl	NaCl	8.0×10^6	9.5×10^4	1.2
NaCl	PB	PB	1.0×10^6	1.3×10^5	13
NaCl	PB	NaCl	1.8×10^6	5.7×10^4	3.2
PB	PB	NaCl	1.6×10^7	5.6×10^6	35
NaCl	NaCl	PB	8.0×10^6	3.2×10^3	0.04

* PB, 0.067 M potassium phosphate buffer (pH 7).

** NaCl, 0.5 M NaCl dissolved in the phosphate buffer. Dose of irradiation was 20 krad.

FIG. 1. Effects of Bovine Serum Albumin, Dextran and Inulin on Radiosensitization by NaCl.

Cells washed and resuspended with phosphate buffer (PB) were mixed with or without 1 M NaCl dissolved with PB. After concentrated solutions of bovine serum albumin, dextran or inulin were dialysed extensively against PB, they were diluted with PB or PB containing NaCl (NaCl-PB) and were mixed with the cell suspension. Cells irradiated in PB ∇ ; NaCl-PB $\times$; bovine serum albumin (200 μ g/ml) $\circ$, $\bullet$; NaCl-PB plus bovine serum albumin (20 μ g/ml) $\odot$; inulin (20 mg/ml) $\triangle$, $\blacktriangle$; dextran (20 mg/ml) $\square$, $\blacksquare$. Open symbols and broken lines, irradiation without NaCl; closed symbols and straight lines, irradiation in the presence of NaCl.

Although the composite effects of NaCl were observed it is clear that cell survivals were substantially reduced by its presence during irradiation. This radiosensitization disappeared when the cells were irradiated in the buffer containing amino acids.^{2,3,7} If intracellular Cl_2^- is responsible for this sensitization, it might be counteracted by cell con-

stituents such as amino acids in the cell. The intracellular concentration of total amino acids in an *E. coli* cell is estimated to be around 10 μ mol/100 mg dry weight of the bacteria⁹ which is more than 10^{-2} M if the single cell volume is calculated as if it were a rod of $0.5 \mu \times 2 \mu$. This concentration is high enough to suggest that at least one of the targets of Cl_2^- might be the cell surface and intracellular Cl_2^- would be effectively counteracted with cell constituents including amino acids. This prediction is supported by the results shown in Fig. 1. The survivors increased substantially when the cells resuspended with the NaCl-buffer were irradiated in the presence of bovine serum albumin (MW = 6.7×10^4 D), dextran (MW = 1.8×10^5 D) or inulin (MW = 5×10^3 D). Since these compounds were mixed with the cell suspension after extensive dialysis, the contribution of low molecular weight constituents in the samples, if any, could be ruled out as a main reason for their effects. It should be pointed out that bovine serum albumin was the most effective at lower concentrations than the other two polysaccharides. This might be mostly due to the difference between their reaction-rate constant with Cl_2^- . It can be expected that the rate constant of bovine serum albumin is much higher than those of the polysaccharides because the rate constants of some amino acids, e.g. tyrosine or tryptophane, are the order of $10^8 \sim 10^9 \text{ M}^{-1} \text{ sec}^{-1}$, while the constant of hexose was less than $10^6 \text{ M}^{-1} \text{ sec}^{-1}$ (data not shown). From the lack of after-effects of the NaCl-buffer irradiated under aerobic conditions at neutral pH,¹¹ long-lived toxic products such as Cl_2 or HClO may not be responsible for this radiosensitization. On the basis of pulse radiolysis studies of the NaCl solution, Jayson *et al.* presented the following mechanism for the production of Cl_2^-

In the phosphate buffer (pH 7) used in this study the equilibrium (4) would be over to the left.¹²⁾ Therefore, a possible involvement of ClOH^- in this radiosensitization must be considered at neutral pH. It is very plausible that a kinetic study for the reactivity of solutes with Cl_2^- by analysing the decay rate of absorption at around 340 nm^{6,7,13)} might include the absorption due to ClOH^- , because Cl_2^- absorption has a maximum at 340 nm whereas ClOH^- has a maximum at 350 nm.¹²⁾ Furthermore, the radical reactions might be more complex and competitive with each other in cell suspensions containing NaCl, amino acids, O_2 and phosphates which are also reactive with OH or H radicals even if phosphates are generally considered to be radiochemically inert.¹⁴⁾ Therefore it is very hard to estimate at present how these radicals contribute the radiosensitization of *E. coli* since there are no data on the reaction-rate constants of these radicals with cell constituents measured individually. However, it seems likely that this radiosensitization might be brought about by injury to the cell membrane and not to be the cell wall because replication of phage, ϕX174 , absorbed previously into the host cells, was substantially inhibited by irradiation in the presence of NaCl (data not shown). Taking into consideration the effects of an ionic strength change and the intricate radical reaction in cell suspension, this radiosensitization might be brought about by the combined effects on the cell membrane and some of which are counteracted by the elimination of Cl_2^- or ClOH^- .

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