Radioprotective Action of Non-Ionic Surfactants on *E. coli* B/r

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When E. coli B/r was irradiated in well air-equilibrated phosphate buffer at 15°C with 40 kR of ⁶⁰Co γ-rays, the presence of many kinds of water soluble non-ionic surfactants during irradiation increased the survival fraction. Tween 80 was the most protective compound among those surfactants tested, and DRF of 2.5 was obtained at 10⁻¹M while 4.0 was obtained for 0.06 M of β-mercaptoethylamine by our assay system. The protective effect of Tween 80 appeared at 10⁻⁴M then increased rather linearly up to 10⁻¹M without reaching plateau. Higher concentration was not investigated because of high viscosity. Long polyoxyethylene chain(s) at the hydrophilic end seems to be effective for the protective action. Possible explanation of the protection mechanism is discussed.

In the course of random screening of radioprotectors for *E. coli* B/r with ⁶⁰Co-γ-irradiation, we have found many water soluble non-ionic surfactants, which have long polyoxyethylene chains in the molecule as hydrophilic group, were radioprotector.

Material and method

Tween and MYRJ were obtained from Kao Atlas Co. (Japan) and Nikkol was obtained from Nikko Chemicals (Japan). Commercial grade was used without further purification. All surfactants used are illustrated in Table 1.

E. coli B/r (CSH) was kindly provided by Dr. H.I. Adler of Oak Ridge National Laboratory and has stocked on nutrient agar slants at 4°C. It has been transferred at every six months.

E. coli B/r grown aerobically 16 hours at 30°C (stational phase) in nutrient broth (Difco) was harvested by centrifugation, washed once with M/15 of Na-phosphate buffer (pH 6.8), then resuspended in the buffer as concentration of about 3×10⁸ cells per millilitre with or without test compounds. Then one part of the suspension was irradiated with ⁶⁰Co γ-rays for 40 minutes at 15°C at the dose rate of 1000 R per minute, and the other

Table 1 List of non-ionic surfactants used in this experiment

Commercial name	Chemical composition	HLB
Tween-80	POE(20)sorbitan monooleate	15.0
Tween-40	POE(20)sorbitan monopalmitate	15.6
Tween-20	POE(20)sorbitan monolaurate	16.7
MYRJ-53	POE(50)stearate	17.9
Nikkol TS-10	POE sorbitan monostearate	14.9
Nikkol BC-40TX	POE cetyl ether	16.4
Nikkol BC-15TX	POE cetyl ether	12.5
Nikkol MYS-40	POE stearate	17.4
Nikkol MYS-55	POE stearate	19.0
Nikkol OP-30	POE octylphenol ether	16.9
Nikkol PBC-34	POE POP cetyl ether	16.4

POE=Polyoxyethylene, POP=Polyoxypropylene

part was incubated for 70 minutes without irradiation to assay their toxicity.

For assay of protective effects they were added to cell suspensions 30 minutes prior to irradiation and incubated for 30 minutes at room temperature with occasional shaking. After the irradiation both irradiated and unirradiated cell suspensions are diluted with the same buffer, each one millilitre of sample is pipetted in three petri dishes with 15 ml of nutrient agar(Difco) then viable cell count was carried out after 16 hours' incubation at 37°C.

Results

Fig. 1 shows radioprotective effect of Tween-80 among various concentration ranges. Radioprotective effect appears at about 10^{-4} M both for Tween-80 and Nikkol TS-10 (Figure is not shown) then increases without

Fig. 1 Survivals of *E. coli* B/r in the presence of various concentration of Tween 80.

Fig. 2 Survivals of *E. coli* B/r with 40 kR of $^{60}\text{Co-}\gamma$ -irradiation at various conditions. (%) are expressed as w/v

reaching plateau like in the case of mercaptoethylamine (MEA)¹⁾.

For unirradiated control at least 90% of cells survive at highest concentration. Comparison of protection of many other surfactants are summarized in Fig. 2. Other kind of solubilizing agents used in biochemical field such as Na-deoxycholate and Digitonin give slight protection at highest concentration could be assayed. Dose reduction factor (DRF) of Tween-80 at highest concentration is roughly calculated as 2.5, while 0.06 M of MEA gives 4.0 by the same assay system.

Discussion

Among those surfactants tested, it could be concluded that sorbitan in their molecule is effective. Compounds which have good solubilization ability²⁾ of oil in water system (means big HLB value³⁾) also give protection.

Mode of action of surfactants are not well known, but when Tween-80 was added just prior to irradiation the protective effect is not changed. This fact will suggest the sensitivity of the bacteria to ionizing radiation largely depends on the condition of surface or near the surface of cells. This protective action is similar to that of observed with MEA which react only to the surface or near the surface of B/r cells to give radioprotective action⁴⁾.

Although many radical scavengers are good protector for radiation degradation of vitamin B₁ in aqueous solution, Tween-80 does not protect this vitamin⁵⁾. This fact could deny the possibility that it would act as radical scavenger in bacterial system. Sulfhydryl-like impurities in Tween-80 are not detected by DTNB reaction⁶⁾. Only possible protecting mechanism concerned to hypotheses which have been proposed so far is anoxia production in bacterial cells⁷⁾. Buffer is previously well air-equilibrated overnight, but surfactants added might form micelles around the oxygen molecules in solution to make anoxic circumstance in bacterial cells. But no experimental evidences are yet obtained to show this possibility.

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要 旨

大腸菌における放射線防護剤

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E. coli B/r を用いた放射線防護剤の研究中に多くの水溶性中性界面活性剤が防護作用をもっていることを見出した。Tween 80 はしらべられた界面活性剤のうちでもっとも強い防護作用を示し、その最高値では2.5の DRF を示した。 β -mercaptoethylamine は0.06 M で 4.0の DRF を示す。Tween 80 の場合は $10^{-4}M$ において防護作用が現われ、 β -mercaptoethylamine の場合のような plateau 濃度を示すことなく、 $10^{-1}M$ まで濃度に比例して防護作用が増加する。水溶性界面活性剤の構造のなかの polyoxyethylene の鎖は親水作用を高めるとともに、この放射線防護作用にも作用していると見られる。作用機作を証明するような実験事実はまだないが、bacteria の表面の状態の変化に起因すると思われる。