Accumulation and Lethal Effect of Tritium (Tritiated Water) in *Rhodopseudomonas spheroides* Under Light-Anaerobic and Dark-Aerobic Conditions

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**Summary.** Nonsulfur purple photosynthetic bacteria, *Rhodopseudomonas spheroides* cells were cultured in medium containing tritiated water (THO) under the light-anaerobic and dark-aerobic conditions. The experimental R value defined as specific activity ratio of organic bound $^3$H to THO in medium was 0.49 and 0.48 for the light-anaerobically grown cells and the dark-aerobically grown cells, respectively. From the relation of R value to number of weight doubling of the cells (n), ratio of experimental R to theoretical R, i.e., $(2^n-1)/2^n$ derived by assuming no isotope effect, was 0.51 and 0.49 on an average for the light-anaerobically grown cells and the dark-aerobically grown cells, respectively. $^3$H-incorporation from THO-medium into the light-anaerobic nongrowing cells was affected by the light intensity and suppressed by adding HgCl$_2$, KCN, and 2,4-dinitrophenol as well as $^3$H-labelling in the dark-aerobic nongrowing cells was affected by oxygen tension and suppressed by adding these metabolic inhibitors. From the fractionation of the lyophilized cells by modified Schneider method, the distribution of exchangeable $^3$H in cold acid-soluble and ether-ethanol-soluble fractions and nonexchangeable $^3$H bound to small molecules and macromolecules was 7.4/25.3/67.3 in the growing cells cultured anaerobically in the THO-medium up to late exponential phase in the light. The distribution in the nongrowing cells incubated anaerobically with the THO-medium for 18 h in the light of 300 and 3,000 lux was 82.1/8.4/9.5 and 58.2/19.2/22.6, respectively. These distributions of $^3$H were changed with growth phase and/or incubation time. On the biological effect of $^3$H-THO for the cells stocked at $-196^\circ$C to accumulate $^3$H-decays, the dark-aerobic nongrowing cells labelled with THO were rather radiosensitive than the dark-aerobically and light-anaerobically grown cells cultured in the THO-medium. The killing efficiencies, i.e., the probability that a single disintegration would be lethal, ranged from $1/200$ to $1/275$ for the above three kinds of cells labelled with THO. The killing efficiencies for *R. spheroides* labelled with THO were similar to that for radiosensitive strain CB13 and wild strain Hfr of *Escherichia coli* labelled with $^3$H-thymidine and stored at $-196^\circ$C.
1. Introduction

Tritiated water (THO) is the major tritium ($^3$H) compound released from nuclear plants into the environment [22]. It is well known that when THO was administered to various organisms, $^3$H was incorporated not only into the intracellular water, but also into the organic materials of the organisms [5, 13]. Since non-sulfur purple photosynthetic bacteria, *Rhodopseudomonas spheroides* cells can grow aerobically in the dark or anaerobically in the light, this bacterium is a suitable organism for studying the metabolism of THO in vivo. In our previous study using the dark-aerobically grown cells [8, 9], specific activity ratio of organic bound $^3$H to environmental THO (experimental R value) was maximum 0.5 and 0.2 in the growing and nongrowing cells, respectively. Further, average experimental R value of nonexchangeable $^3$H in the nucleic acids and these mononucleotides in the growing cells ranged from 0.4–0.6 like the R value of the total cell materials. In the present study, the metabolism of $^3$H-THO in the light-anaerobically grown cells and the dark-aerobically grown cells was compared by the observations of bacterial growth in the medium containing THO (THO-medium), the distribution of bound $^3$H in organic materials and the effect of metabolic inhibitors for $^3$H-incorporation. The results indicated that the extent of the bacterial growth inhibition became larger as THO-concentration of medium was higher. Further, $^3$H-incorporation from THO into the light-anaerobic nongrowing cells was affected by light intensity and suppressed by adding HgCl$_2$, KCN, and 2,4-dinitrophenol (DNP) as well as $^3$H-incorporation in the dark-aerobic nongrowing cells was affected by oxygen tension and inhibited by adding these metabolic inhibitors. From the fractionation of lyophilized cells, it was shown that in the light-anaerobically grown cells 92.6% of total bound $^3$H incorporated into the growing cells was nonexchangeable form but in the nongrowing cells 58.2–82.1% of total bound $^3$H was exchangeable form. Our studies on the biological effect of $^3$H-THO for the cells stored at $-196^\circ$C indicated that the dark-aerobic nongrowing cells labelled with THO were rather radiosensitive than both the light-anaerobically grown cells and the dark-aerobically grown cells cultured in the THO-medium.

2. Materials and Methods

2.1. Bacterial Strain and Cultivation Condition

*R. spheroides* originally obtained from Van Niel was grown anaerobically in the light and aerobically in the dark in medium S of Lascelles [14] as described previously [6, 7].

For observations of the effect of THO on bacterial growth and $^3$H-incorporation under growth condition, about $10^8$ cells grown light-anaerobically was inoculated in 10 ml of the THO-medium in a test tube with silicone screw cap of 10 ml capacity and cultivated semi-anaerobically at $30^\circ$C in the light of 3,000 lux.