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Oxidative stress on anaerobes

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A strict anaerobe, *Prevotella melaninogenica* is highly sensitive to oxidative stress. Oxidative stress such as exposure to oxygen or addition of hydrogen peroxide, increased 8-hydroxydeoxyguanosine (8OHdG), a typical of oxidative DNA damage, and decreased the bacterial cell survival rate. We could detect the generation of reactive oxygen species in *P. melaninogenica* after exposure to oxygen. UVA irradiation also increased 8OHdG in the bacterium. On the other hand, such oxidative stress did not increase 8OHdG in a facultative anaerobe. These findings suggest that *P. melaninogenica* is a suitable material to study the biological effects of oxidative stress, to evaluate antioxidants, and to study the effects of oxygen or reactive oxygen species on molecular evolution.

Key words : anaerobes, 8-hydroxydeoxyguanosine, oxidative DNA damage, reactive oxygen species, *Prevotella melaninogenica*

INTRODUCTION

Reactive oxygen species (ROS) are considered to be playing important roles on the development of various disorders such as arteriosclerosis, cancer and neurodegenerative diseases [1]. ROS may also have intimate connection with aging or molecular evolution [2, 3]. However, it is hard to investigate the effects of ROS on aerobes because they are equipped with efficient defense systems against ROS. Strict anaerobes could not survive under the ambient condition and must be highly sensitive to oxidative stress. Here we investigated the response of anaerobes to oxidative stress using 8-hydroxydeoxyguanosine (8OHdG), a typical of oxidative DNA damage, as a marker.

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MATERIALS AND METHODS

Preparation of Bacterial cells. *Prevotella melaninogenica* (which is a strict anaerobe and showed neither superoxide dismutase (SOD) nor catalase activities), *Bacteroides fragilis* (which is a strict anaerobe and had both SOD and

catalase activities) and *Salmonella typhimurium* (which is a facultative anaerobe and had both SOD and catalase activities) were grown and prepared as described previously [4].

Exposure to oxidative stress. Bacterial cell suspension was exposed to oxygen gas for 30 sec., and then incubated for the indicated time at 37 ℃. The indicated concentrations of hydrogen peroxide were added to the cell suspension, then the cell suspension was incubated for the indicated time at 37 ℃ [4]. The cell suspension was also exposed to UVA for 10 min at 0 ℃ (365 nm, ~ 700 kJ/m^2).

Determination of 8OHdG. DNA was extracted from the bacterial cells and digested to nucleosides with enzymes under an anaerobic condition as described previously [5]. The nucleosides were separated by an HPLC system, then 8OHdG was detected by electrochemical detection and deoxyguanosine (dG) was detected by UV absorption as described before [6]. The ratio of 8OHdG/100,000 dG was calculated.

Determination of cell survival. Bacterial cell survival was determined by colony formation after the exposure to oxidative stress [4].

Determination of ROS. ROS was determined by flow cytometry with dichlorofluorescin diacetate (DCFH-DA) or hydroethidine (HE) [7]. ROS was also determined by ESR with α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN) as described [8].

Results and Discussion

As shown in Figure 1, exposure to oxygen increased 8OHdG and decreased cell survival [4] only in *P. melaninogenica*. Oxygen seemed to be converted to ROS in *P. melaninogenica*, because exposure of oxygen to DNA did not increase 8OHdG.

As shown in Figure 2, hydrogen peroxide increased 8OHdG and decreased cell survival only in *P. melaninogenica*.

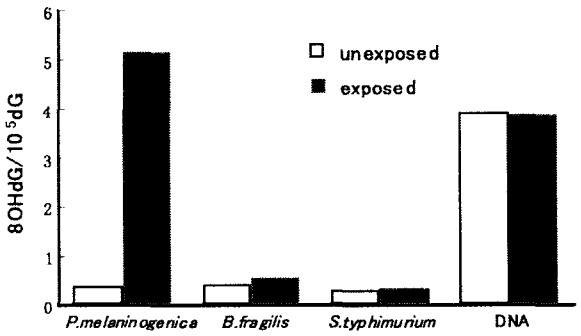

Figure 1. 8OHdG induction by O_2 exposure

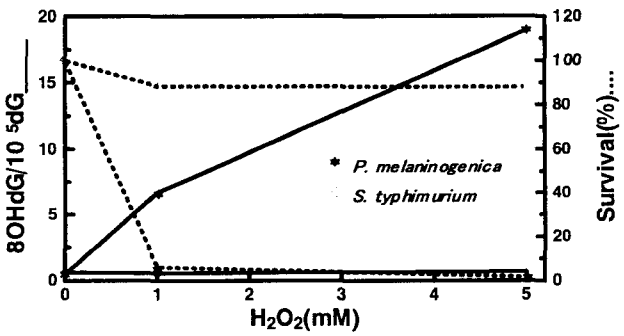

Figure 2. Effects of H_2O_2 on anaerobes

These results indicate that *P. melaninogenica* is highly sensitive to oxidative stress. However, B. fragilis, which is also a strict anaerobe, was not so sensitive as *P. melaninogenica*, probably because *B. fragilis* had both SOD and catalase activities.

Figure 3a shows the generation of ·OH in *P. melaninogenica* after exposure to oxygen. Figure 3b shows the generation of hydrogen peroxide and superoxide in *P. melaninogenica* after exposure to oxygen.

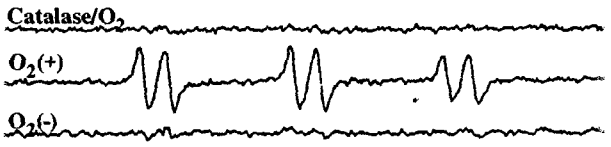

Figure 3a. ESR detection of ·OH radical derived signal with POBN in *P. melaninogenica*.

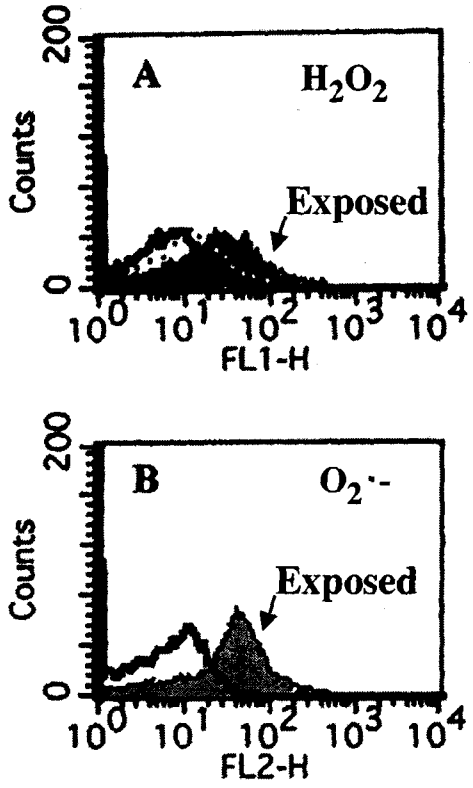

Figure 3b. Flow cytometric detection of hydrogen peroxide and superoxide with DCFH-DA(A) and HE(B) in *P. melaninogenica*. The dotted line in (A) indicates the flow cytogram of the exposed sample in the presence of catalase.

We could not detect the increase of ROS in *B. fragilis* or *S. typhimurium* after exposure to oxygen. These data indicate that even in a strict anaerobe oxygen was utilized and the oxygen was converted to ROS, which might induce cell death and 8OHdG.

In the case of *B. fragilis* and *S. typhimurium* ROS might be generated but the ROS might be efficiently removed by SOD and catalase.

UVA irradiation also increased 8OHdG in *P. melaninogenica* (Figure 4). Riboflavin had no effects on the induction of 8OHdG by UVA. UVA irradiation also increased 8OHdG in *B. fragilis* but not in *S. typhimurium*. When UVA was irradiated in the presence of oxygen, the increase in 8OHdG was enhanced (data not shown). The mechanism of 8OHdG induction by UVA irradiation is under study.

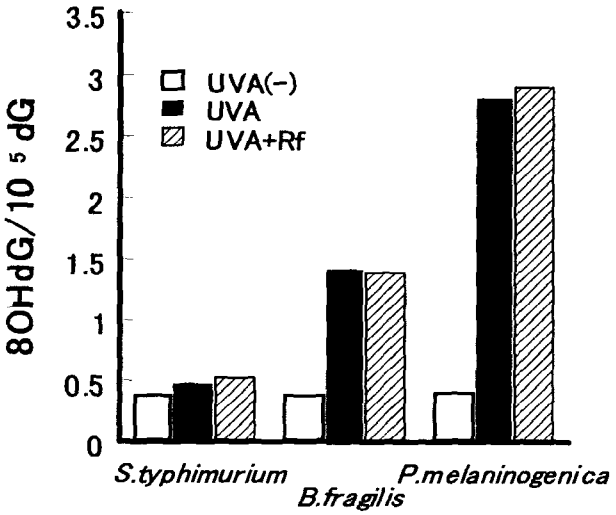

Figure 4. Induction of 8OHdG by UVA irradiation in the absence or in the presence of riboflavin.

From these findings we consider that *P. melaninogenica*, a strict anaerobe without SOD or catalase activities, is highly susceptible to ROS and that it could be used as a model to look at the effects of ROS on biological systems.

Because *P. melaninogenica* increased its oxidative damage after oxidative stress considerably, it could also be used to evaluate the function of antioxidants [4].

We further studied the fate of the induced 8OHdG in *P. melaninogenica*. In the case of mammalian cells the induced 8OHdG was removed rapidly from the DNA [9]. However, in *P. melaninogenica* the induced 8OHdG was not removed (Figure 5).

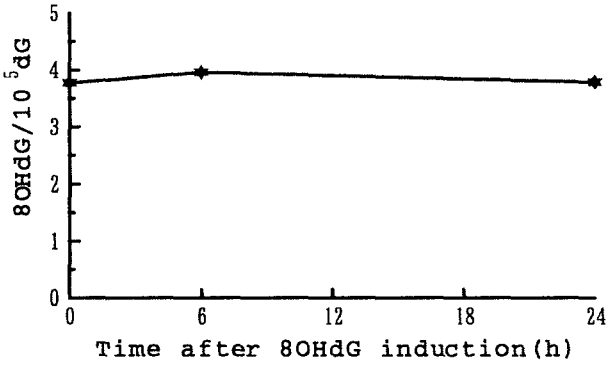

Figure 5. Time course of the induced 8OHdG levels in *P. melaninogenica*.

We consider that *P. melaninogenica* could be used to study the mutagenicity of oxygen-induced 8OHdG with other oxidative DNA damage and that it may show us the effects of oxygen on molecular evolution.

In conclusion, *P. melaninogenica* is an interesting and useful tool to study the effects of oxygen and ROS on the biological system.

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