Light Irradiation Is a Factor in the Bactericidal Activity of Silver-Loaded Zeolite

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Silver loaded zeolite (Ag-Z) was previously found to have effective bactericidal activity against *Escherichia coli*. To understand the mechanisms of bactericidal activity of Ag-Z, role of light irradiation was focused and investigated in this study. In this study, we focused on light irradiation. Antibacterial assay and spectroscopic study revealed that light irradiation enabled Ag-Z to reduce dioxygen to form a reactive oxygen species, which led to bactericidal activity. These results indicate that the onset of bactericidal activity can be controlled by light irradiation.

Key words antibacterial activity; silver; light irradiation; zeolite; *Escherichia coli*

Sterilization is an important process for sanitation and hygiene. Recently, inorganic antibacterial materials have become more attractive and are used in various situations.[3)] For example, they can be used in the production of a wide variety of goods, as they have higher resistance to heat, chemicals and radiation than organic compounds. Among inorganic antibacterial materials, silver-loaded materials are particularly attractive due to their effective antibacterial activity.[4—6)] We previously confirmed the antibacterial activity of silver-loaded zeolite (Ag-Z) in deionized water at relatively short contact times (less than 1 h).[7)] The introduction of silver ions into zeolite (crystalline alumino silicate) allows the reduction of dissolved dioxygen to form reactive oxygen species.

The aim of this study was to identify the factors necessary for Ag-Z to activate dissolved oxygen. The light was focused in this paper as an exogenous factor stimulating the reaction. To investigate the contribution of the light irradiation in the antibacterial activity of Ag-Z, antibacterial assay was performed under shaded conditions. The formation of reactive oxygen species was studied by spectroscopy, as they were considered to contribute to the antibacterial activity.[7)]

The present results may represent a valuable contribution to material science because antibacterial activity may be controlled by the factor studied.

Experimental

Chemicals Reactive oxygen species were observed by using cytochrome C (Sigma, MO, U.S.A.) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO, Wako Pure Chem. Ind. Ltd., Osaka, Japan).

Deionized water (>17 MΩ cm) used in this study was prepared using the Puric-S system (Organo Co., Ltd., Tokyo, Japan).

Preparation of Silver-Loaded Zeolite (Ag-Z) Ag-Z was prepared by the ion-exchange method, as described previously.[7)] Sodium-type zeolite (Na-Z, faujasite structure, Mizusawa Chemical Co., Ltd.) was used as the host compound. For antibacterial assay, 0.1 g of Ag-Z was fixed with polyvinylidene fluoride (PVF).

Antibacterial Assay *Escherichia coli* NIHJ JC2 was used to assess the bactericidal activity of Ag-Z. Bactericidal activity was estimated by counting viable cell after each treatment.

Bactericidal activity was measured under either light-irradiated or shaded conditions. The latter was achieved by covering the vessel containing the bacterial suspension and Ag-Z-PVF with aluminum foil. The suspension (300 cm^3) containing *E. coli* (*ca.* 10^7 cfu cm^{-3}) and Ag-Z-PVF was stirred with a magnetic stirrer at 37 °C. Ag-Z-PVF was then carefully added to the suspension, with particularly care taken to prevent light from entering the vessel under shaded conditions. Sampling times were 5, 10, 15, 20, 30, 45 and 60 min after the addition of Ag-Z-PVF. Viable cell counts were determined visually as the number of colonies per plate in serial 10-fold dilutions.[8)]

UV/VIS Measurement UV/VIS spectra of cytochrome C solution were measured using a Shimadzu UV-1600PC UV–Visible spectrophotometer. Cytochrome C solution was 50 mM phosphate-buffered saline including 0.1 mM ethylenediaminetetraacetic acid (EDTA, Kanto Chem. Co., Inc., Tokyo, Japan) and 5 mM cytochrome C (Sigma, MO, U.S.A.). Cytochrome C solution containing Ag-Z (0.1 g) was stirred for 30 min with or without light irradiation and was filtered (ϕ 0.45 nm). Spectra were measured at 550 nm.

UV/VIS diffuse reflection spectra of powder compounds were measured on a Shimadzu UV-2400PC UV–Visible spectrophotometer, and barium sulfate was used as a standard. Powder of cation-loaded zeolite was fixed on sample tray and deionized water (100 μl) was added for the aqueous condition.

ESR Measurement Deionized water (0.5 ml) was poured into a small vessel, and then 0.05 ml of DMPO was added. Ag-Z powder (0.1 g) was added to the DMPO solution and the suspension was stirred vigorously. The obtained suspension was injected into a quartz crystal capillary tube, the end of which was sealed with ESR-silent clay. The capillary was placed in a TE-mode cavity using a sample holder and spectra were measured by JEOL-RE1X spectrometer worked at X-band (9.4 GHz). The ESR parameters were follows: center field was 334.488 mT; sweep width was +/− 7.5 mT; scan rate was 4 mT min^{-1}; field modulation frequency was 100 kHz; filed modulation amplitude was 0.1 mT; and time constant was 0.1 s. After addition of Ag-Z, the reaction vessel and ESR measurement system were light-irradiated or shaded, as necessary.

Results and Discussion

The contribution of light irradiation to the antibacterial activity of Ag-Z was investigated. Figure 1 shows the time course of viable cell counts under irradiated and shaded conditions. Under light-irradiated conditions, almost all cells lost the ability to form colonies after only 5 min of contact. However, changes in viable cell number were scarcely observed under shaded conditions, with viable cell counts being similar to those under control conditions (no Ag-Z added). It means also that adsorption of *E. coli* on zeolite was negligible. These results suggest that light irradiation was necessary to induce bactericidal activity against *E. coli*.

In a previous report, bactericidal activity of Ag-Z was observed with the formation of reactive oxygen species.[7)] Spec-

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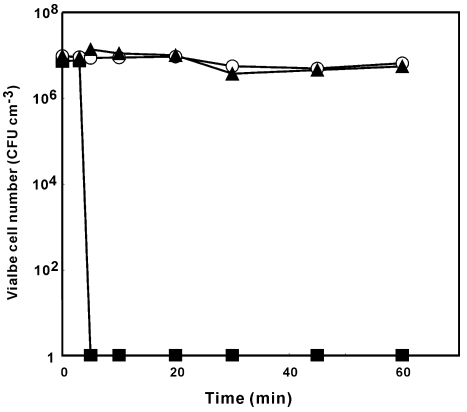

Fig. 1. Changes in Viable Cell Count after the Addition of Cation-Loaded Zeolite

Open circles: control (Na-Y at irradiated condition); closed triangles: Ag-Z under shaded conditions; closed squares: Ag-Z under irradiated conditions.

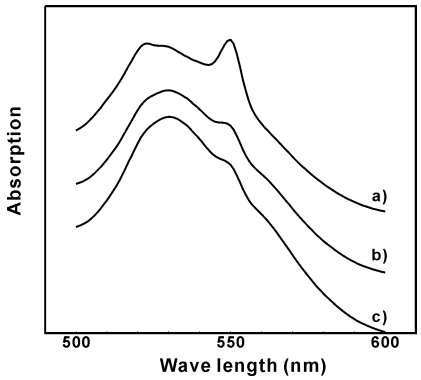

Fig. 2. UV/VIS Spectra of Ag-Z Suspension Containing Cytochrome C

a) Irradiated conditions, b) shaded conditions, c) cytochrome C only (control). Conditions: cytochrome C (50 mM); 200 μl; supernatant, 2 ml.

troscopic study was carried out to investigate the formation of superoxide anions, as they are formed by one electron reduction of dioxygen and lead to formation of the hydroxyl radicals that play an important role in bactericidal activity.[9] Furthermore, the contribution of light in this reduction was investigated.

The UV/VIS spectrum of the suspension containing Ag-Z and cytochrome C was measured in order to investigate the formation of superoxide anions after light irradiation (Fig. 2). The spectra of the superoxide anion generation system ($H_2O_2+FeSO_4$) indicated absorption at around 550 nm (data not shown).

The spectrum obtained under shaded conditions was similar to that obtained for controls. On the other hand, apparent absorption at around 550 nm was observed under light-irradiated conditions. These results suggest that light irradiation, which is necessary for bactericidal activity, induced the reduction of dioxygen to form superoxide anions.

In order to investigate whether the formation of hydroxyl radicals requires light irradiation, ESR spectra were measured, as hydroxyl radicals are thought to be an important bactericidal species.[10,11] A solution containing only DMPO was measured as a control.

Under shaded conditions, the spectrum was similar to that of the control (Fig. 3b). Irradiated conditions gave a spectrum showing a 1 : 2 : 2 : 1 quartet (Fig. 3a). This is typical of

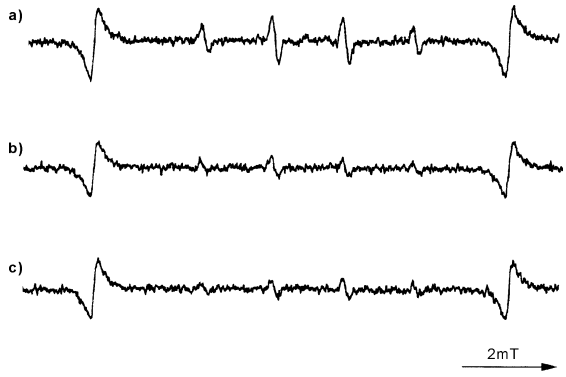

Fig. 3. ESR Spectra of Ag-Z

a) Ag-Z under irradiated conditions, b) Ag-Z under shaded conditions, c) DMPO only (control).

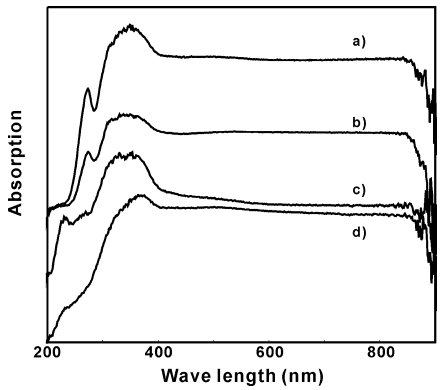

Fig. 4. Diffuse Reflectance UV/VIS Spectra in Cation-Loaded Zeolite

a) Ag-Z under dry conditions, b) Ag-Z under aqueous conditions, c) Na-Z under dry conditions, d) Na-Z under aqueous conditions.

hydroxyl radical-DMPO adducts, and indicates the formation of hydroxyl radicals. Therefore, the formation of hydroxyl radical was confirmed to be dependent on light irradiation.

Diffuse reflectance UV/VIS spectra of Ag-Z were measured to investigate the effect of loading silver ions into zeolite (Fig. 4). The spectra of sodium loaded zeolite (Na-Z) were measured as a reference.

For Ag-Z, absorption around 270 nm was observed. This absorption peak was not observed for Na-Z. Therefore, this absorption peak was due to the introduction of silver ions. The diffuse reflectance UV/VIS spectra were also measured under aqueous conditions, as Ag-Z is immersed in deionized water during bactericidal assay. Aqueous conditions were achieved by the addition of deionized water (100 μl) to a powder sample set in a sample tray. The spectrum was similar to that under dry conditions.

Rabani *et al.* reported that this absorption peak in Ag-Z was due to superoxide anions.[12] Spectral results confirmed that absorption at 270 nm was present under both aqueous conditions and dry conditions. This suggests that loading silver ions into zeolite allows the formation of reactive oxygen species from dioxygen dissolved in the small amounts of water in the zeolite lattice, and prolongs to the lifespan of these reactive oxygen species. This characteristic is very important for the use of Ag-Z as a bactericidal material.

The results obtained in this study confirm the necessity of light irradiation for the bactericidal activity of Ag-Z. This study thus indicates that it is possible to control the bacterici-

dal activity of Ag-Z by selectively applying light irradiation, and may encourage the wider usage of antibacterial materials such as Ag-Z because reactive oxygen species can be generated at requested time and requested place.

References and Notes

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