Influence of the mixing ratio on antibacterial characteristics of MgO–ZnO solid solution in two phase coexistence region

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Though occurrence of the antibacterial activity on some oxides, MgO and ZnO, is well-known, it is not clear what the mixing ratio among solid solutions affects antibacterial activity. In present work, change in antibacterial activity with the mixing ratio of two-type solid solutions, Mg1–xZnxO with cubic structure and Zn1–xMgxO with hexagonal structure, was studied. After MgO and ZnO powders were mixed with the molar ratio (MgO/ZnO) of 0.44, 1.22 and 1.86, the solid solutions were obtained by heating at 1200°C for 5 h in air. By X-ray diffraction measurement, it was found that two-type solid solutions of cubic and hexagonal structure were formed at the molar ratio of 1.22–0.44. The formation amount of cubic-type solid solution increased with increasing the molar ratio. At the molar ratio of 1.86, cubic-type solid solution was formed without any hexagonal-type. In three powder samples of the obtained solid solutions, antibacterial characteristics were examined by colony count method, using Staphylococcus aureus and Escherichia coli. In the case of the mixture of cubic- and hexagonal-type solid solutions, antibacterial activity enhanced with increasing the formation amount of cubic-type solid solution. The powder sample with a single phase of cubic-type solid solution showed strongest antibacterial activity in three powder samples. As mentioned above, the mixing ratio of two-type solid solutions with cubic and hexagonal structure was found to affect antibacterial activity.

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1. Introduction

Conventional antibacterial materials, such as chlorine disinfectant and silver ion, have been used in water treatment process and food industry. However, the utilization of these materials has some problems. For example, chlorine disinfectant has been reported to produce either trihalogenated compounds or carcinogenic products by reacting with organic materials. Also, since a large amount of silver ion is known to cause chronic toxicity, much precaution is required for the use of the silver ion from viewpoint of human health. Therefore, it is important to develop new antibacterial materials substituting for chlorine disinfectant and silver ion.

Recently, zinc oxide (ZnO) and magnesium oxide (MgO) powders have received great attentions as new antibacterial materials, which consists of mineral element and shows antibacterial activity in small amount of these oxides without the presence of light. Not only MgO and ZnO but also MgO–ZnO solid solution is known to exhibit the antibacterial activity. Yamamoto et al. studied the change in antibacterial activity with an increase in doping amount of ZnO in a single phase region of MgO–ZnO solid solution with cubic structure. They clarified that the increase in doping amount of ZnO in the solid solution resulted in the reduction of antibacterial activity. A single phase of solid solution with cubic structure is formed above the molar ratio (MgO/ZnO) of 1.86. According to the phase diagram of MgO–ZnO system, the formation of two-type solid solutions with cubic and hexagonal structure is recognized in the range from 0 to 1.86. However, it is not yet clear what antibacterial activity is expected to affect the mixing ratio of two-type solid solutions.

MgO–ZnO solid solution powders were formed by heating the powder mixtures with different molar ratios. Effect of the mixing ratio of cubic- and hexagonal-type solid solutions on antibacterial activity was studied in present work, based on the antibacterial evaluation of colony count method.

2. Experimental

2.1 Powder characterization

ZnO (Kanto Chemical, Co., Purity; 99.9%) and MgO (Ube Chemical IND, Ltd. Purity; 99.9%) powders were used as starting materials. These powders were mixed in mortar in different molar ratios (MgO/ZnO) of 0.44, 1.22 and 1.86. The mixtures were heated at 1200°C for 5 h in air, in order to obtain three MgO–ZnO solid solution powders. As reference materials against solid solutions, ZnO and MgO powders were obtained at identical heat-treatment condition. X-ray diffraction measurement (XRD; RIGAKU Co., RAD-C SYSTEM) of the powder samples obtained was carried out in order to confirm the formation of solid solution. The formation ratio (Cubic/Hexagonal) of cubic- and hexagonal-type solid solution obtained was determined by dividing a (200) peak area of cubic-type by a (101) peak area of hexagonal-type.

Specific surface area of the powder samples was determined by using the adsorption isotherms of N2 at −196°C (BET; BELJAPAN, INC. BELSORP-mini). It is essential to measure pH value of the solution containing the powder samples, because
high alkalinity is known to affect antibacterial activity. In order to examine the pH value, the powder samples were dispersed into distilled water at the powder concentration of 0.63 g dm⁻³. After keeping the dispersed solution for 1 h, the pH value of the solution was measured.

2.2 Antibacterial test

Escherichia coli 745 (hereafter, *E. coli*) and Staphylococcus aureus 9779 (hereafter, *S. aureus*) were used as test bacteria. The test bacteria were suspended into an LB medium, which contains 0.5% yeast extract (Becton, Dickinson and Co.), 1% bacitrepone (Becton, Dickinson and Co.) and 1% sodium chloride (WAKO PURE CHEMICAL IND., LTD, purity: 99.9%) (NaCl). The medium was cultured at 36°C for 48 h on a reciprocal shaker and rinsed four times with sterile water. Finally, the bacterial suspension was diluted at a concentration of 10⁷ cfu dm⁻³ (cfu; Colony Forming Unit), and then mixed with sterile water containing powder samples in the concentration ranging from 0.08 to 1.25 g dm⁻³.

Antibacterial test was initiated by shaking the suspension at 36°C on a reciprocal shaker. After pipetting the suspension of 10⁴ dm⁻³ for specified time, the suspension was spread on a nutrient agar (NA, Eiken Chemical, Co.) against *E. coli* and a pearl-core plate count agar (PPCA, Eiken Chemical, Co.) against *S. aureus*. The colonies formed on the agar were counted after the incubation at 36°C for 24–48 h without the presence of light. Antibacterial activity of the powder samples was assessed by calculating the ratio \( \frac{N/N_0} \) between the viable bacterial counts \( N \) (cfu dm⁻³) for specified time and the initial counts (control) of bacteria \( N_0 \) (cfu dm⁻³).

3. Results and discussion

3.1 Powder characterization

Sample code, molar ratio (MgO/ZnO), formation phase, and formation ratio of MgO–ZnO solid solution are summarized in Table 1. Figure 1 shows XRD patterns of the powder samples obtained by heating at 1200°C for 5 h in air. (a) ZnO, (b) ZM1, (c) ZM2, (d) ZM3 and (e) MgO.

### Table 1. Sample Code, Molar Ratio, Formation Phase, and Formation Ratio of the Solid Solutions Used in this Study

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Molar ratio (MgO/ZnO)</th>
<th>Formation phase</th>
<th>Formation ratio (Cubic/Hexagonal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZM1</td>
<td>0.44</td>
<td>Cubic + Hexagonal</td>
<td>0.25</td>
</tr>
<tr>
<td>ZM2</td>
<td>1.22</td>
<td>Cubic + Hexagonal</td>
<td>2.7</td>
</tr>
<tr>
<td>ZM3</td>
<td>1.86</td>
<td>Cubic</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 1. XRD patterns of the powder samples obtained by heating at 1200°C for 5 h in air. (a) ZnO, (b) ZM1, (c) ZM2, (d) ZM3 and (e) MgO.

3.2 Antibacterial activity

In colony count method, the powder sample when the survival ratio changes with a steep decrease at the specified time can be understood to have stronger antibacterial activity. Figures 2(a) and (b) show change in survival ratio of *S. aureus* and *E. coli*, respectively, using three solid solutions and original powders suspended at the powder concentration of 0.63 g dm⁻³. In the case of *S. aureus* (see Fig. 2(a)), the survival ratio reduced with increasing time in all powder samples. The survival ratio on ZM3 exhibited reduction in a shorter time than other powder samples. The reduction of the survival ratio on ZM1 was found to be gentler than that on ZM2. These results are presumed that antibac-
terial activity of three solid solution powders enhanced with increasing the formation amount of cubic-type solid solution. In E. coli (see Fig. 2(b)), the reducing behavior of the survival ratio was comparable with those of S. aureus.

In the comparison of the survival ratio at specified time between MgO and ZnO powders, the reduction of the survival ratio on MgO was larger than that on ZnO, irrespective of the kind of bacteria. That is, antibacterial activity of MgO with cubic structure was found to be stronger than that of ZnO with hexagonal structure.

Regarding the formation amount of cubic-type solid solution in two phase coexistence region, it is reasonable that the antibacterial activity of two-type solid solutions is dependent on those of cubic-type solid solution having stronger antibacterial activity. On antibacterial activity of MgO with cubic structure, it has been reported that super-oxide anion (O$_2^-$) as an antibacterial agent was generated from its surface. Thus, the occurrence of antibacterial activity on cubic-type solid solution should be attributed to the generation of O$_2^-$.

Figures 3(a) and (b) show change in survival ratio of S. aureus and E. coli in each powder concentration of ZM3 with a single phase of cubic-type solid solution. The survival ratio reduced with increasing powder concentration; that is, the antibacterial activity of cubic-type solid solution enhanced with increasing the powder concentration. From the result, the enhancement of antibacterial activity with increasing the formation amount of cubic-type solid solution is presumed to be due to the increase of generating amount of O$_2^-$.

On the other hand, the antibacterial activity of ZnO with hexagonal structure has been reported that the generation of hydrogen peroxide (H$_2$O$_2$) as an antibacterial agent was detected on its surface. Consequently, chemical species contributing to antibacterial activity of cubic-type solid solution is different from that of hexagonal-type solid solution. It has been reported that self-dismutation reaction of O$_2^-$ proceeds rapidly in an aqueous solution with the pH value ranging from 7 to 9, as expressed by the following Eq. (1).$

2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \tag{1}$

Since the pH values of ZM1, ZM2 and ZM3 were 8.4, 8.4 and 8.7, respectively, self-dismutation reaction of O$_2^-$ might occur in this work. However, the antibacterial activity of ZnO powders that occurred due to the generation of H$_2$O$_2$ was weakest in all powder samples. Assuming that H$_2$O$_2$ generated by self-dismutation reaction of O$_2^-$ contributed to antibacterial activity, antibacterial effect of H$_2$O$_2$ might be less than that of O$_2^-$, depending on the amount of O$_2^-$. Therefore, the reason that the antibacterial activity enhanced with increasing the formation amount of cubic-type solid solution is presumed to be due to the increase of the generating amount of O$_2^-$. In comparison of the survival ratio between E. coli and S. aureus, the ratio of S. aureus was less than that of E. coli that
is, antibacterial action towards \textit{S. aureus} is stronger than that towards \textit{E. coli}. It has been reported that the sensitivity on \textit{E. coli} to chemical species such as \textit{O}_2\textsuperscript{-} is not identical with that on \textit{S. aureus}\textsuperscript{20}. The reason that the solid solution showed strong antibacterial activity against \textit{S. aureus} rather than \textit{E. coli} is presumed to be due to the high sensitivity to \textit{O}_2\textsuperscript{-}.

4. Summary

\textit{MgO–ZnO} solid solution powders were prepared by heating the mixture with molar ratios (\textit{MgO}/\textit{ZnO}) of 0.44, 1.22 and 1.86. By XRD measurements, it was found that the molar ratios of 0.44 and 1.22 resulted in two-type solid solutions with cubic and hexagonal structure. And the formation amount of cubic-type solid solution increased with increasing the molar ratio. A single phase of cubic-type solid solution was obtained at the molar ratio of 1.86. In antibacterial tests, it was clarified that the activity enhanced with increasing the formation amount of cubic-type solid solution; that is, antibacterial activity of \textit{MgO–ZnO} solid solution in two phase coexistence region was confirmed to be dependent on the mixing ratio. The powder sample with a single phase of cubic-type solid solution showed the strongest antibacterial activity in all powder samples, irrespective of the kind of bacteria.

References