# Antibacterial Activity of Resin-Containing Triethylenetetramine Side Chains and/or Thiol Groups–Metal Complexes

TAKAMASA NONAKA,* YASUKO UEMURA, KOICHIRO ENISHI, and SEIJI KURIHARA

Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kurokami 2-39-1, Kumamoto-shi 860, Japan

SYNOPSIS

Four chelating resins containing triethylenetetramine side chains and/or thiol groups were made from macroreticular 2,3-epithiopropyl methacrylate, styrene–divinylbenzene (DVB), or methyl methacrylate–DVB copolymer beads, and then the resins bearing metal ions such as $Ag^+$, $Cu^{2+}$, and $Zn^{2+}$ were made. The antibacterial activity of the resins bearing metal ions against *Escherichia coli* (*E. coli*) or *Staphylococcus aureus* (*S. aureus*) was investigated. The resins containing thiol groups showed the higher adsorption capacity for silver ions than for other metal ions. The resins, which contain both triethylenetetramine side chains and thiol groups, bearing silver ions (RE-TTA-Ag) exhibited high antibacterial activity against bacteria, especially *E. coli*, without the residual silver ions in water after contacting with bacteria. The activity of the RE-TTA-Ag did not decrease even after reusing several times. © 1996 John Wiley & Sons, Inc.

## INTRODUCTION

Chlorine or many soluble disinfectants are used for sterilizing water. However, soluble disinfectants have a problem of residual toxicity of the agents, even when suitable amounts of the agents are used. Recently, to prevent such a residual toxicity of the agents, insolubilized agents having an antibacterial activity have been developed.[1-7] On the other hand, it is well known that silver ions kill bacteria in water.[8] Therefore, when silver ions are added into a solution containing bacteria, bacteria are killed. However, an excess of silver ions that were not used to kill bacteria remain in a solution. Although it is said that silver ions are not harmful to human beings, the residual concentration of silver ions in the wastes is limited to the concentration below 50 ppb by law in the USA.

In order to kill bacteria effectively and leave no silver ions in a solution, a required amount of silver ions should be added gradually into a suspension containing bacteria and then be in contact with bacteria. If solids bearing silver ions are placed into a solution containing bacteria, it is considered that silver ions released from the solids kill bacteria such as *Escherichia coli* (*E. coli*) or *Staphylococcus aureus* (*S. aureus*) and their antibacterial activity will last over long time, when silver ions are released gradually from the solids. So far, it has been reported that a few materials such as zeolites or polyacrylonitrile-based activated carbon fibers, etc., bearing silver ions have such an antibacterial activity. However, the adsorption ability of silver ions on such materials is not particularly strong; consequently, they have a tendency to release into water.[9-11] We selected the chelating resins, which have high adsorption ability for silver ions, as solids bearing silver ions.

In this article, we describe the preparation of chelating resins, which have high adsorption ability for metal ions, particularly, silver ions, release of metal ions from the resins, and their antibacterial activity against *E. coli* or *S. aureus*.

* To whom correspondence should be addressed.

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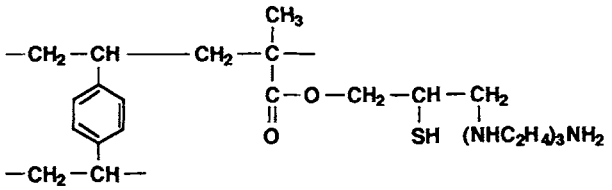
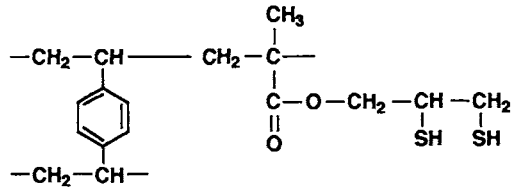
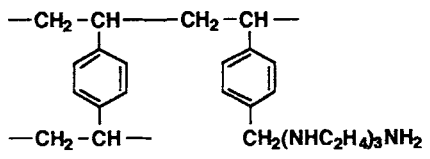


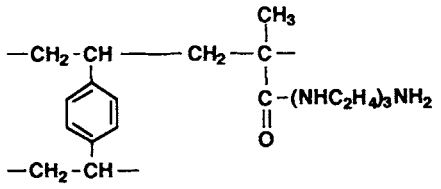
(RE-TTA)



(RE-KSH)



(RCS-TTA)



(RM-TTA)

**Figure 1** Various resins used in this study.

## EXPERIMENTAL

### Materials

2,3-Epithiopropylmethacrylate (ETMA) was synthesized and purified by a method reported earlier. $\alpha,\alpha'$-azobisisobutylonitrile was recrystalized from ethanol. Other chemicals were of reagent grade.

### Preparation of Macroreticular Chelating Resins

Macroreticular ETMA–divinylbenzene (DVB) copolymer beads were synthesized by suspension polymerization in the presence of 2,2,4-trimethylpentane as diluent. Four chelating resins having thiols and/or triethylenetetramine side chains, the structures of which are shown in Figure 1, were prepared as follows.

### RE-TTA and RE-KSH

The RE-TTA and RE-KSH resins were obtained by treating the ETMA-DVB copolymer beads (32–60 mesh) with triethylenetetramine (TTA) in 1,4-dioxane at 90°C for 8 h and with ethanolic potassium hydrogen sulfide at 50°C for 1 h, respectively.[12,13]

### RCS-TTA

Styrene–DVB (St-DVB) (10 mol %) copolymer bead (RS) was synthesized by suspension polymerization in the presence of 2,2,4-trimethylpentane, and RCS was prepared by following treatment of the RS with chloromethylether. RCS-TTA was prepared by amination of RCS with TTA in benzene at 100°C for 2 h.[14,15]

### RM-TTA

Methyl methacrylate–DVB (MMA-DVB) (10 mol %) copolymer beads (RM) was synthesized by suspension polymerization in the presence of 2,2,4-trimethylpentane, and RM-TTA was prepared by aminolysis of the RM with TTA at 175°C for 5 h.[16]

### Preparation of Resins Bearing Various Metal Ions

0.5 g of the resins and 50 $cm^{33}$ of buffered metal ion solution were placed in a glass-stoppered 100 $cm^3$

**Table I Anion Exchange Capacity and Adsorption Capacity of Resins for Metal Ions**

| Resin | Anion Exchange Capacity (meq/g-R) | Adsorption Capacity (meq/g-R) | | |
|---|---|---|---|---|
| | | Ag | Cu | Zn |
| RE-TTA | 4.12 | 2.75 | 1.32 | 0.62 |
| RCS-TTA | 5.44 | 2.42 | — | — |
| RM-TTA | 5.05 | 2.02 | — | — |
| RE-KSH | — | 3.52 | — | — |

Weight of resin: 0.50 g; shaking at 30°C for 24 h. Loading solution: 0.1 mol/$dm^3$ $AgNO_3$; 0.03 mol/$dm^3$ $CuCl_2$; 0.03 mol/$dm^3$ $Zn(NO_3)_2$ solution.

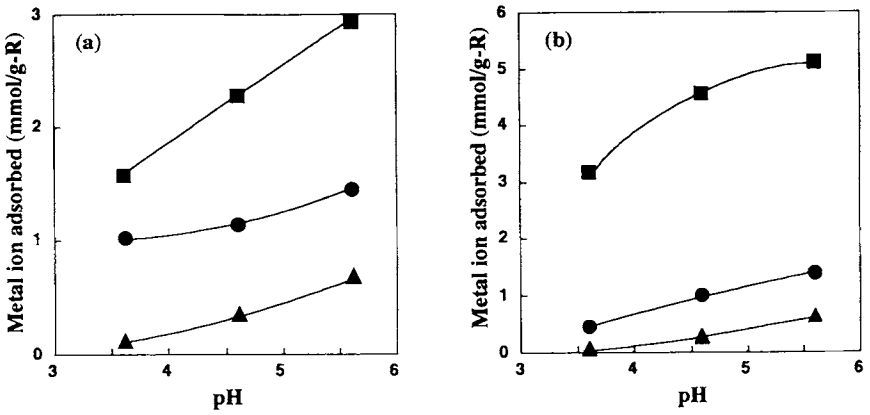



**Figure 2** Effect of pH on the adsorption of various metal ions: Metal ion: (■) $Ag^+$; (●) $Cu^{2+}$; (▲) $Zn^{2+}$. Conditions are as follows. Resin: RE-TTA (a) RE-KSH (b) 0.125 g. Initial concentration of the metal ion: 0.01*M* (50 mL). In buffer solution: $CH_3COOH$–$CH_3COONa$. Shaking at 30°C for 24 h.

Erlenmeyer flask. Then the mixture was allowed to stand at 30°C for 24 h with occasional shaking. The amount of metal ions adsorbed on the resins was calculated by determining the concentration of metal ions in the supernatant by atomic absorption spectroscopy.

## Organism and Growth Conditions

The bacteria used in this study were *E. coli* (IFO 3301) and *S. aureus* (IFO 13276), which were obtained commercially from the Institute for Fermentation, Osaka.

One loopful of bacteria cells on culture medium was inoculated into 100 $cm^3$ of 0.5 wt % nutrient broth and cultivated at 30°C overnight (ca. 16 h), then 1 $cm^3$ of the precultivated suspension was placed into 100 $cm^3$ of fresh 0.5 wt/vol % nutrient broth and cultivated at 30°C for 3.5–4 h. Then the cells in the cultured cell suspension were collected by centrifugation at 10,000 rpm for 15 min and washed with sterile deionized water, then they were again suspended in water and were stabilized at 30°C for 1–2 h under shaking. By diluting the cell suspension with sterile deionized water, cultured cell suspension containing ca. $10^7$–$10^8$ cells/mL was prepared for each strain and used for antibacterial tests.

## Contact of Resins Bearing Metal Ions with Bacteria

A desired amount of resins bearing metal ions was placed in 50 $cm^3$ Erlenmeyer flask; then 10 $cm^3$ of cell suspension and subsequent 10 $cm^3$ of water were

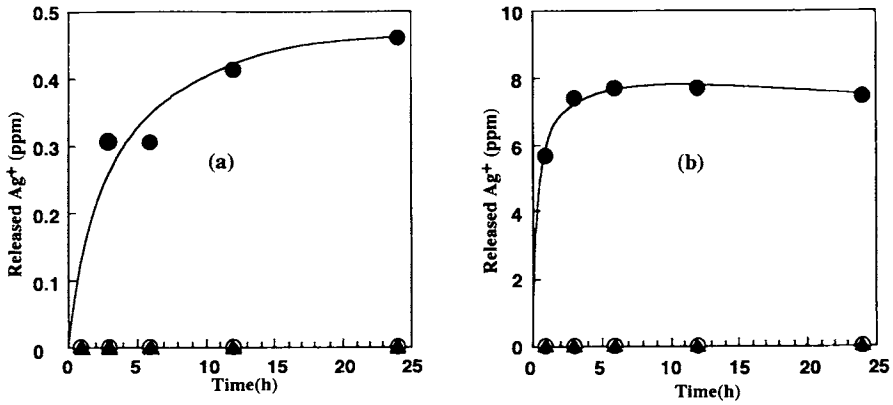



**Figure 3** Concentration of released $Ag^+$ from RE-TTA-Ag (a) and RE-KSH-Ag (b) in deionized water (20 mL). Symbols and $Ag^+$ content (meq/g-R) for (a) are as follows: (△) 0.08; (○) 0.16; (▲) 0.31; (●) 0.62. Symbols and $Ag^+$ content (meq/g-R) for (b) are as follows: (○) 0.80; (△) 1.61; (●) 3.22. Weight of resin is 0.10 g; shaking at 30°C.

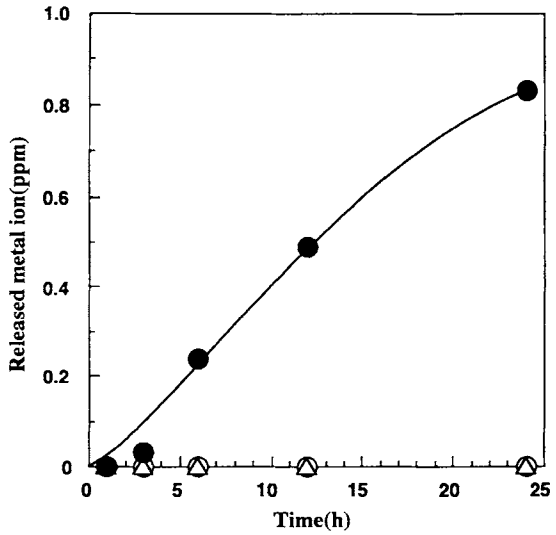



**Figure 4** Concentration of released metal ion from RE-TTA-Ag, Cu, and Zn in deionized water (20 mL). Symbols and resins are as follows: (○) RE-TTA-Ag; (△) RE-TTA-Cu; (●) RE-TTA-Zn. Metal ion content is 0.16 (meq/g-R); weight of resin is 0.10 g; shaking at 30°C.

added into the flask, and the flask was shaken at 30°C for desired time.

### Measurement of Viable Cell Number After Contacting with Resins

After contacting the resins bearing metal ions with bacteria suspension for prescribed time, 1 $cm^3$ of the bacteria suspension was pipetted out from the flask, and 9 $cm^3$ of sterile water were added to this suspension. The suspension was diluted several times, and 0.1 $cm^3$ of the diluted suspension was spread on an agar plate made of nutrient agar. The plate was kept at 37°C for 15–24 h, and the numbers of viable cells were calculated from those of the colonies formed on the plate.

### Measurement of Absorption Capacity of Bacteria Cells for Metal Ions

Metal ion solutions and bacteria suspensions were mixed and shaken at 30°C for 6 h, and then residual concentration of metal ions in the supernatant was measured by atomic absorption spectroscopy and the absorption capacity (mmol/cell) was calculated.

## RESULTS AND DISCUSSION

### Adsorption of Metal Ions on the Resins

Anion exchange capacity and adsorption capacity of the RE-TTA, RCS-TTA, RM-TTA, and RE-KSH for silver ion ($Ag^+$) were measured by batch method. The results are shown in Table I. The RE-KSH had no anion exchange capacity because it had thiol groups but no triethylenetetramine side chains.

The order of anion exchange capacity of these resins is as follows: RCS-TTA > RM-TTA > RE-TTA. However, of three resins containing triethylenetetramine side chains, the RE-TTA had the highest adsorption capacity for $Ag^+$ in spite of its lowest anion exchange capacity. In Table I, the adsorption capacity of the RE-TTA for the copper ion ($Cu^{2+}$) and the zinc ion ($Zn^{2+}$) is also listed. The RE-TTA was found to adsorb more $Ag^+$ than $Cu^{2+}$ or $Zn^{2+}$. It was also found that the RE-KSH having dithiol groups per monomer unit had a higher adsorption capacity for $Ag^+$ than the RE-TTA. These results indicate that thiol groups in the resins participated significantly in the adsorption of $Ag^+$.

Figure 2(a,b) show the pH dependence of the adsorption capacity of the RE-TTA and RE-KSH for $Ag^+$, $Cu^{2+}$, and $Zn^{2+}$, respectively. In the whole pH range studied, the order of adsorption capacity of the RE-TTA and RE-KSH for metal ions is as follows: $Ag^+ > Cu^{2+} > Zn^{2+}$. The adsorption capacity of the RE-KSH for $Ag^+$ was about twice that of the RE-TTA, while the adsorption capacity of both resins for $Cu^{2+}$ and $Zn^{2+}$ was almost the same. These results indicate that silver ions were adsorbed strongly on the RE-TTA or RE-KSH having thiol groups in solutions of such pHs.

### Leakage of Metal Ions from the Resins

In order to study the leakage of metal ion from the resins bearing metal ions, the RE-TTA and RE-

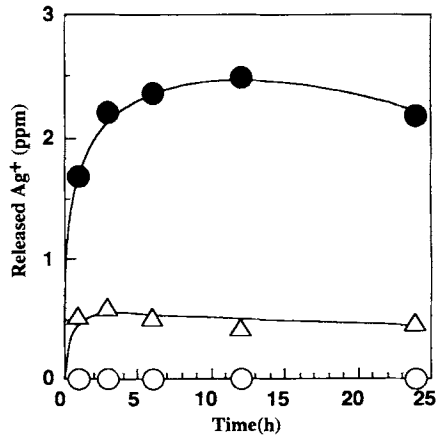



**Figure 5** Concentration of released $Ag^+$ from RE-TTA-Ag, RCS-TTA-Ag, and RM-TTA-Ag in deionized water (20 mL). Symbols and resins are as follows: (○) RE-TTA-Ag; (△) RCS-TTA-Ag; (●) RM-TTA-Ag. $Ag^+$ content is 0.16 meq/g-R; weight of resin is 0.10 g; shaking at 30°C.

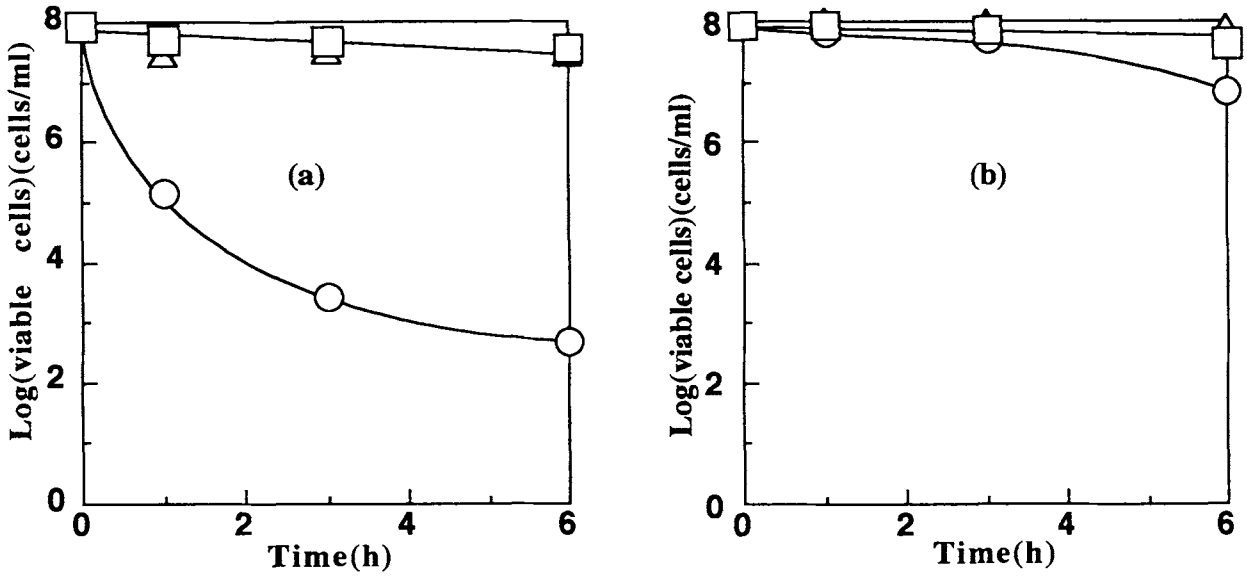



**Figure 6** Changes in viable cell number of *E. coli* (a) or *S. aureus* (b) after contacting with RE-TTA or RE-TTA-Ag.

| Symbol | Resin | Final pH (a) | Final pH (b) | $Ag^+$ Concentration (ppm)[a] (a) | $Ag^+$ Concentration (ppm)[a] (b) |
|---|---|---|---|---|---|
| □ | Blank | 6.40 | 6.28 | — | — |
| △ | RE-TTA | 6.89 | 6.54 | 0 | 0 |
| ○ | RE-TTA-Ag | 5.91 | 5.98 | 0 | 0 |

[a] Concentration of $Ag^+$ in water after experiment. Suspension of bacteria: 20 mL (water). Shaking at 30°C. Weight of resin: 0.10 g. $Ag^+$ content: 0.16 (meq/g R).

KSH bearing different amount of $Ag^+$ were first prepared; then they were soaked under shaking in deionized water for desired times and the leakage of $Ag^+$ from the resins was investigated. The results are shown in Figure 3(a,b). No release of $Ag^+$ from the RE-TTA bearing $Ag^+$ of less than 0.31 meq/g-R was observed, while the significant amount of $Ag^+$ was released from the RE-TTA bearing $Ag^+$ of 0.62 meq/g-R, as shown in Figure 3(a). In the case of the RE-KSH, the release of $Ag^+$ from the RE-KSH bearing different amounts of $Ag^+$ (0.80–3.22 meq/g-R) was measured [Fig. 3(b)]. No release of $Ag^+$ from the RE-KSH bearing $Ag^+$ of less than 1.61 meq/g-R was observed, while the release of $Ag^+$ from the RE-KSH bearing $Ag^+$ of 3.22 meq/g-R was observed.

The release of metal ions from the RE-TTA bearing 0.16 meq/g-R of $Ag^+$, $Cu^{2+}$, or $Zn^{2+}$ was also measured (Fig 4). No release of metal ions, except for $Zn^{2+}$, was observed.

The release of $Ag^+$ from the RE-TTA, RCS-TTA, and RM-TTA bearing $Ag^+$ of 0.16 meq/g-R was measured (Fig. 5). The RCS-TTA and RM-TTA have triethyleneteramine side chains, but no thiol groups. The order of ease of release of $Ag^+$ from the resins is as follows: RM-TTA > RCS-TTA > RE-TTA = 0. These results indicate that the order of difficulty of release of $Ag^+$ from the resins is as follows: RE-TTA > RCS-TTA > RM-TTA.

To kill bacteria effectively, the resins have to gradually release a required amount of $Ag^+$ and to outlast its antibacterial activity for a long time. Therefore, from the results described above, we selected the RE-TTA that has suitable adsoption capacity and adsorption strength as solid material bearing silver ions.

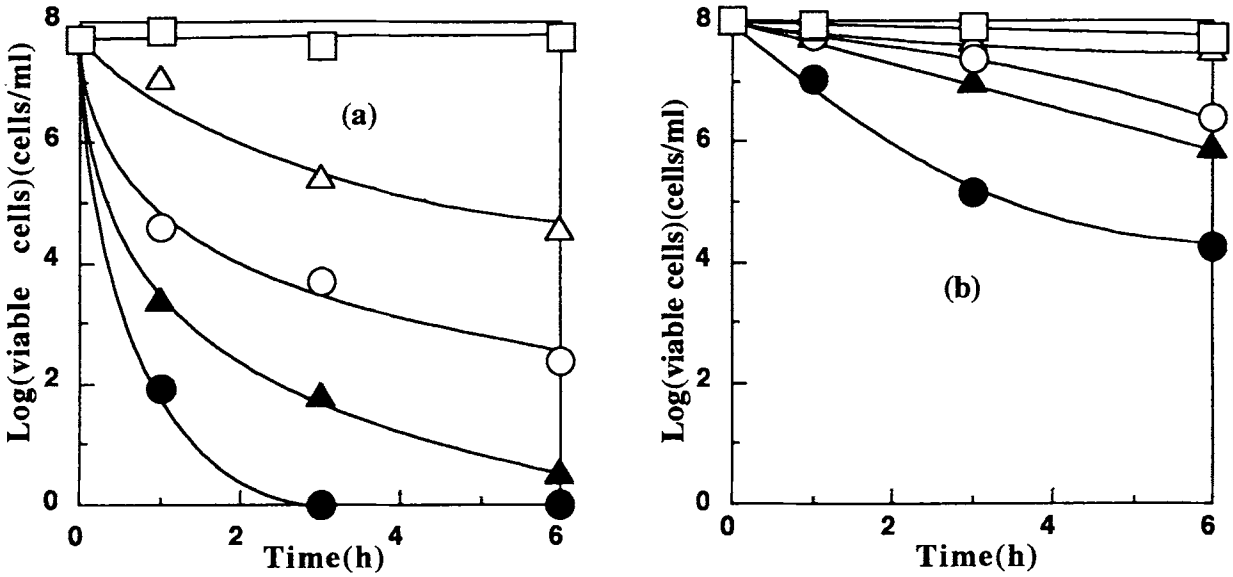



**Figure 7** Changes in viable cell number of *E. coli*1 (a) or *S. aureus* (b) after contacting with the resins (RE-TTA-Ag) having different amount of $Ag^+$.

| Symbol | Resin | $Ag^+$ Content (meq/g-R) | Final pH (a) | Final pH (b) | $Ag^+$ Concentration (ppm)[a] (a) | $Ag^+$ Concentration (ppm)[a] (b) |
|---|---|---|---|---|---|---|
| □ | Blank | — | 6.61 | 6.75 | — | — |
| △ | RE-TTA-Ag (I) | 0.08 | 6.63 | 5.85 | 0 | 0 |
| ○ | RE-TTA-Ag (II) | 0.16 | 6.52 | 5.92 | 0 | 0 |
| ▲ | RE-TTA-Ag (III) | 0.31 | 6.46 | 5.85 | 0 | 0 |
| ● | RE-TTA-Ag (IV) | 0.62 | 5.83 | 4.96 | 0 | 0.34 |

[a] Concentration of $Ag^+$ in water after experiment. Suspension of bacteria: 20 mL (water). Shaking at 30°C. Weight of resin: 0.10 g.

## Antibacterial Activity of the RE-TTA and RE-TTA-Ag

The antibacterial activity of the RE-TTA and the resin (RE-TTA-Ag) bearing $Ag^+$ of 0.16 meq/g-R against *E. coli* and *S. aureus* was investigated. The results are shown in Figure 6. The RE-TTA exhibited no antibacterial activity against both bacteria. However, the RE-TTA-Ag exhibited high antibacterial activity against *E. coli,* while it exhibited a little activity against *S. aureus.* In both experiments, No $Ag^+$ was observed in the suspension after contacting with the resins for 6 h as shown below Figure 6. The pHs of the suspension after contacting with the RE-TTA and RE-TTA-Ag for 6 h are also listed in the caption for Figure 6. The pHs are in the range from 5.91 to 6.89. We have previously reported that the solutions of such pHs exhibited by itself no antibacterial activity against bacteria such as *E. coli* and *S. aureus.*[17]

The antibacterial activity of $AgNO_3$, against *E. coli* was also investigated. $Ag^+$ solutions of above 0.1 ppm showed antibacterial activity against *E. coli* (ca. $10^8$ cells/mL) in suspension.

The antibacterial activity of the RE-TTA bearing different amount of $Ag^+$ was investigated against *E. coli* and *S. aureus* (Fig. 7). The antibacterial activity increased with increasing amounts of $Ag^+$ in the resins against both bacteria, and the activity against *E. coli* of the resins bearing the same amount of $Ag^+$ was observed to be higher than that against *S. aureus.* In this experiment, the released $Ag^+$ was not found in the *E. coli* suspension after contacting with the resin after 6 h. However, released $Ag^+$ was observed only after contacting the RE-TTA-Ag (0.62

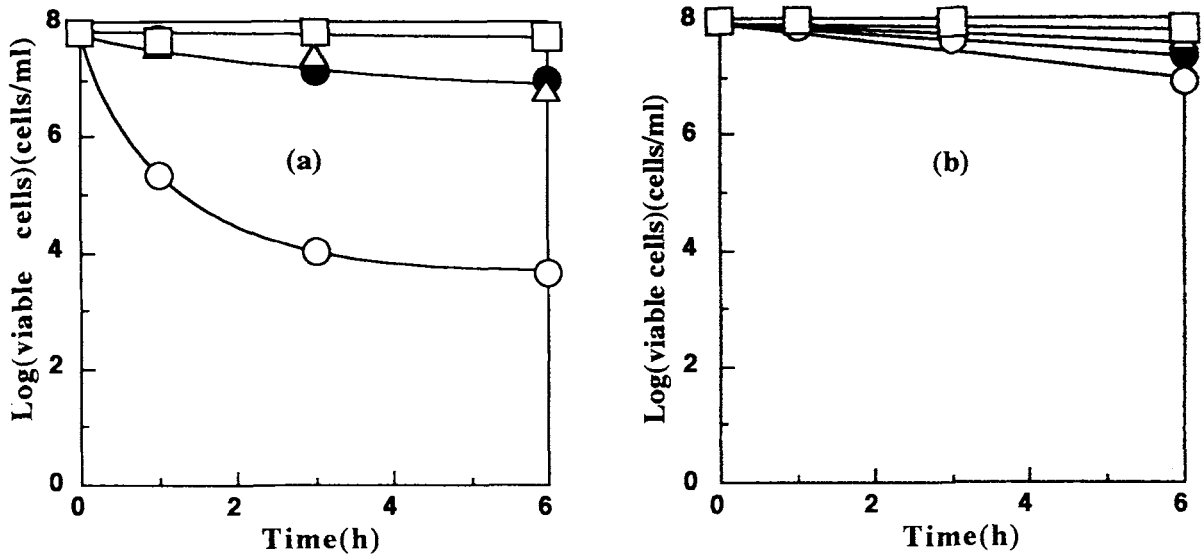



**Figure 8** Changes in viable cell number of *E. coli* (a) or *S. aureus* (b) after contacting with the resins (RE-TTA) having different metal ions.

| Symbol | Resin | Final pH (a) | Final pH (b) | Metal Ion Concentration (ppm)[a] (a) | Metal Ion Concentration (ppm)[a] (b) |
|---|---|---|---|---|---|
| □ | Blank | 6.50 | 6.19 | — | — |
| ○ | RE-TTA-Ag | 5.95 | 6.21 | 0 | 0 |
| △ | RE-TTA-Cu | 5.60 | 5.31 | 0 | 0 |
| ● | RE-TTA-Zn | 6.05 | 5.84 | 0.20 | 0.26 |

[a] Concentration of metal ion in water after experiment. Suspension of bacteria: 20 mL (water). Shaking at 30°C. Weight of resin: 0.10 g. Metal ion content: 0.16 (meq/g-R).

meq/g-R) with *S. aureus* suspension as shown in the caption for Figure 7.

The antibacterial activity of the RE-TTA-metal (0.16 meq/g-R) bearing various metal ions ($Ag^+$, $Cu^{2+}$, or $Zn^{2+}$) was investigated against *E. coli* and *S. aureus* (Fig. 8). Prior to this experiment, the antibacterial activity of $Ag^+$, $Cu^{2+}$, and $Zn^{2+}$ solution of 0.5 ppm against *E. coli* was investigated. $Ag^+$ and $Cu^{2+}$ exhibited high activity; however, $Zn^{2+}$ exhibited no activity. Of these resin–metal complexes, the RE-TTA-Ag exhibited the highest antibacterial activity against *E. coli* while the RE-TTA-Cu and RE-TTA-Zn exhibited very low activity, as shown in Figure 8(a). Those three RE-TTA-metals showed only very low antibacterial actity against *S. aureus* [Fig. 8(b)]. And a small amount of released $Zn^{2+}$ was observed in both suspensions only after contacting with the RE-TTA-Zn. In Figure 4, we found that $Zn^{2+}$ of 0.26 ppm was released from the RE-TTA-Zn bearing $Zn^{2+}$ of 0.16 meq/g-R after shaking for 6 h. Therefore, this result indicates that most of the released $Zn^{2+}$ could not be absorbed into *S. aureus* cells. This means that no antibacterial activity of $Zn^{2+}$ solution of 0.5 ppm is due to no absorption of $Zn^{2+}$ into bacteria.

The RE-KSH-Ag (0.80meq/g-R) also showed high antibacterial activity against *E. coli*, and no leakage of $Ag^+$ was observed after contacting the resins with bacteria (Fig. 9). However, in this case, the RE-KSH had to bear $Ag^+$ of more than 0.80 meq/g-R to exhibit high antibacterial activity.

We found that the RE-TTA-Ag exhibited higher antibacterial activity against *E. coli* than *S. aureus*. In order to clarify this difference of antibacterial activity against the two bacteria, the absorption of $Ag^+$ and $Cu^{2+}$ into the two kinds of bacteria cells was measured. The results are shown in Table II. It was found that both $Ag^+$ and $Cu^{2+}$ could be absorbed more largely into *E. coli* than *S. aureus*. Furthermore, the amount of $Ag^+$ absorbed into *E. coli* was

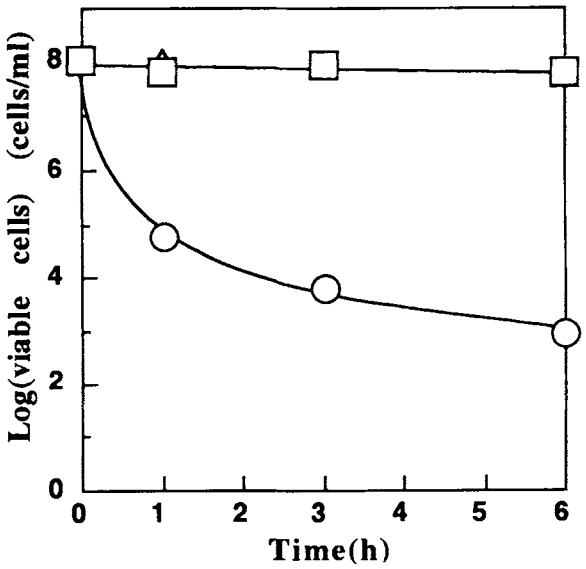



**Figure 9** Changes in viable cell number of *E. coli* after contacting with RE-KSH or RE-KSH-Ag.

| Symbol | Resin | Final pH | $Ag^+$ Concentration (ppm)[a] |
|---|---|---|---|
| □ | Blank | 6.53 | — |
| △ | RE-KSH | 6.03 | 0 |
| ○ | RE-KSH-Ag | 6.01 | 0 |

[a] Concentration of $Ag^+$ in water after experiment. Suspension of *E. coli*: 20 mL (water). Shaking at 30°C. Weight of resin: 0.10 g. $Ag^+$ content: 0.80 (meq/g-R).

about three times that absorbed into *S. aureus*. This result corresponds to the higher antibacterial activity of the RE-TTA-Ag against *E. coli* than *S. aureus*. However, it is not clear at present why more $Ag^+$ could be absorbed into *E. coli* than *S. aureus*.

## Recycled Use of the RE-TTA-Ag and RE-KSH-Ag

The RE-TTA-Ag (0.16 meq/g-R) and RE-KSH-Ag (0.80 meq/g-R) were reused several times to kill bacteria. The results are shown in Figure 10. From these results, it was found that the antibacterial activity of the RE-TTA-Ag did not decrease even after contacting six times with bacteria suspension; however, that of the RE-KSH-Ag decreased rapidly. This results suggest that $Ag^+$ ions were released gradually into bacteria suspension from the RE-TTA-Ag. The remarkable decrease in antibacterial activity of RE-KSH-Ag is due to the difficulty of release of $Ag^+$ from the resins.

**Table II Absorption of Metal Ions with Bacteria**

| Metal Ion | Amount of Metal Ion Absorbed (mmol/cell) × $10^{12}$ | |
|---|---|---|
| | *E. coli* | *S. aureus* |
| $Ag^+$ | 1.05 | 0.33 |
| $Cu^{2+}$ | 0.79 | 0.25 |

Shaking at 30°C for 6 h. Initial concentration of metal ion: 5 ppm.

## Mechanism of Antibacterial Activity of the Resin-Ag Complexes Against Bacteria

As mentioned before, it is known that $Ag^+$ can easily combine with enzymes; therefore, it exhibit antibacterial activity. However, the exact mechanism of its activity is not clear at present. Considering the mechanism that was proposed at this time, the tentative mechanism is as follows.

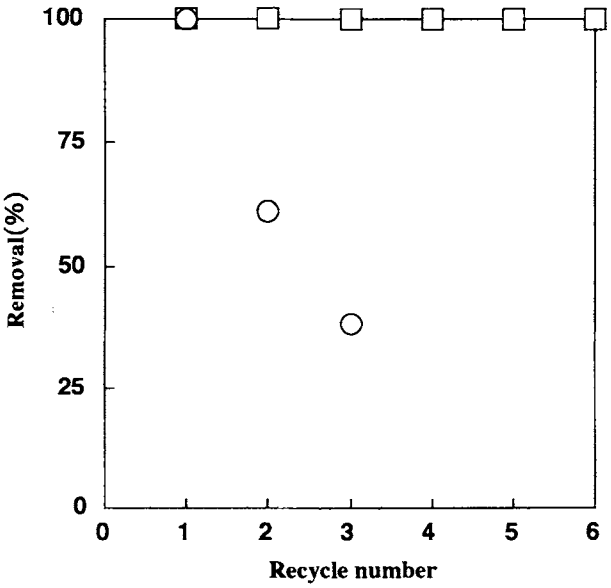



**Figure 10** Changes in removal (%) of *E. coli* after recycling RE-TTA-Ag or RE-KSH-Ag.

| Symbol | Resin | $Ag^+$ Content (meq/g-R) | Weight of Resin (g) | Suspension of *E. coli* (mL) |
|---|---|---|---|---|
| □ | RE-TTA-Ag | 0.16 | 0.25 | 50 |
| ○ | RE-KSH-Ag | 0.80 | 0.10 | 20 |

Initial viable cell number: about $10^8$ cells/mL; shaking at 30°C.

When the resins bearing $Ag^+$ contacted with bacteria suspension, $Ag^+$ on the resins is released, and it penetrated into bacteria, then combines with enzyme in the cell membrane. This results in the inhibition of enzymatic reaction and the death of bacteria.

## CONCLUSIONS

1. The resins that had high adsorption capacity for silver ions could be prepared.
2. The resins, which contain both triethylenetetramine side chains and thiol groups, bearing silver ions (RE-TTA-Ag), exhibited high antibacterial activity against bacteria without the residual silver ions in water after contacting with bacteria.
3. The RE-TTA-Ag had higher antibacterial activity against. *E. coli* than *S. aureus.*
4. The RE-TTA-Ag exhibited high antibacterial activity even after they were used several times.

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