Correlation between ζ-Potential of a Cell in a New Cationic Disinfectant Solution and Minimum Inhibitory Concentration of the Disinfectant

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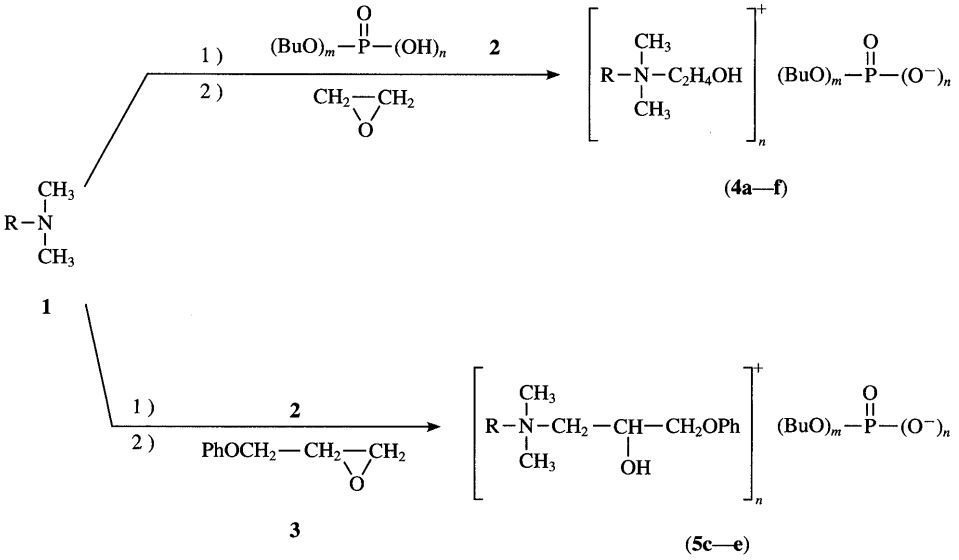
In the previous paper, we described the synthesis of new quaternary ammonium salts with antimicrobial activity: *N*-alkyl-*N*-2-hydroxyethyl-*N*,*N*-dimethylammonium butyl phosphate (4) and *N*-alkyl-*N*-(2-hydroxy-3-phenoxy)-propyl-*N*,*N*-dimethylammonium butyl phosphate (5). In this study, we examined the relationship between the *MIC* (minimum inhibitory concentration) of these new cationic disinfectants, 4 (six compounds) and 5 (three compounds), and the ξ-potential against *Escherichia coli* in solutions of these compounds. The *MIC* values of these disinfectants against *E. coli* were highly correlated with the concentration that induced electric charge inversion (ζ-potential = 0) of *E. coli* from negative to positive.

Keywords ζ-potential; adsorption; disinfectant; minimum inhibitory concentration; quaternary ammonium salt

Quaternary ammonium salts are cationic surfactants with strong bactericidal activity and are widely used as disinfectants. We have described the synthesis and the minimum inhibitory concentration (*MIC*) of many cationic disinfectants that have an alkyl phosphate anion as a counter ion.[1–3] The antibacterial properties and the mechanism of the antibacterial action of these disinfectants have been explored in many studies.[4–16]

An electrokinetic phenomenon occurs on a solid-liquid interface when surfactants are adsorbed onto the solid surface, since the surfactant molecules contain hydrophilic and lipophilic groups. The use of the ζ-potential method has been reported in determining the adsorption behavior of surfactants onto gelatin layers formed on a glass surface,[17,18] and in determining the electrophoretic behavior of leucocytes,[19] red blood corpuscles,[20] and microcapsules containing aqueous solutions of polyelectrolytes.[21]

In an aqueous solution, there are two forces on the interface between the surface of a cell and a cationic surfactant. One is an electrical interaction between the surface electric charge of the cell and the electric charge of the cationic surfactant, and the other is the van der Waals force between two molecules. Since the surface of a cell that is suspended in water usually has a negative charge, if a change in the electric charge on the surface of a cell is detected in a solution of cationic surfactant (disinfectant) by any method, it would indicate that the cationic surfactant has penetrated into or been adsorbed onto the cell. In the present study, we measured the ζ-potential of *Escherichia coli* in disinfectant solution (Chart 1) to examine its relationship to the *MIC* of these



2: butyl acid phosphate (monoester/diester mole ratio=49/51) monoester ($m=1, n=2$) diester ($m=2, n=1$)
3: phenylglycidyl ether
a: R=C_8H_{17} **b**: R=$C_{10}H_{21}$ **c**: R=$C_{12}H_{25}$ **d**: R=$C_{14}H_{29}$ **e**: R=$C_{16}H_{33}$ **f**: R=$C_{18}H_{37}$

Chart 1

TABLE I. *MIC* of New Disinfectants to Organism

Organism	No.[a]	*MIC* (μg/ml)									
		BAC	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**5c**	**5d**	**5e**
E. coli	92411	200	>1600	800	200	400	800	>1600	400	800	1600

The *MIC* was defined as the lowest concentration that inhibited visible growth after 48 h of incubation at 37 °C. The *MIC* was tested by the agar dilution method using Muller-Hinton Agar (Nissui Seiyaku Co., Ltd.). BAC: benzalkonium chloride. *a*) The organism had been isolated from a patient in Shinshu University Hospital.

disinfectants (Table I) against this bacterium.

Results and Discussion

Correlation between ζ-Potential of *E. coli* and *MIC* against *E. coli* The change in ζ-potential that occurred just after the application of different concentrations of disinfectant to a suspension of *E. coli* was measured by electrophoresis at pH 7.5, which is the pH generally employed for disinfectant solutions. The relation between the logarithm of the concentration of disinfectant (C μg/ml) and the ζ-potential (mV) is plotted in Fig. 1.

The ζ-potential of *E. coli* without disinfectant lies between −38 and −41 mV. The ζ-potential of *E. coli* changes from negative to positive with increasing concentration of disinfectant. The turning point ($\zeta = 0$) is the electric charge inversion. The ζ-potential change occurred at the lowest concentration in the case of **4f**, which has the longest alkyl chain group, and at a higher concentration in the case of **4c** which has a shorter alkyl chain group.

These results suggest that **4c**, **5c** and BAC (benzalkonium chloride) may permeate fairly readily through the cell membrane, and bind electrostatically with intracellular anionic groups, as well as with anionic groups on the cell surface. As a result, the ζ-potential increases with increasing adsorption onto the cell surface, and the ζ-potential continuously changes from negative to positive. However, **4f**, **5d**, and **5e** cannot penetrate the cell membrane because they have long alkyl chain groups and high hydrophobicity, suggesting that van der Waals adsorption takes precedence over electrostatic binding. These surfactants align their alkyl chain groups on the cell surface, finally forming a cationized surface which provides an electrostatic shield against the cationic surfactants in solution, hindering penetration into the cell membrane, so that inversion of the D-potential occurs with low concentrations of cationic surfactants with longer alkyl chain groups (Fig. 1).

Accordingly, we considered that the concentration of cationic surfactant that penetrated the cell increased linearly in the order of **4c**, **4d**, **4e**, and **4f** among homologues of **4**, and in the order of **5c**, **5d**, and **5e** among the homologues of **5**. In particular, there was no change of the ζ-potential until log 1.8 for **4c** and there was a rapid increase after log 1.8. The reason for this is considered to be that **4c** penetrates the cell until log 1.8, and the electric charge in the cell is neutralized by **4c**, which is adsorbed onto its surface by electrostatic binding; at this point **4c** is unable to penetrate the cell any more. The phenomenon of the increasing ζ-potential between log 2.5 and log 2.8 would be due to electrical saturation

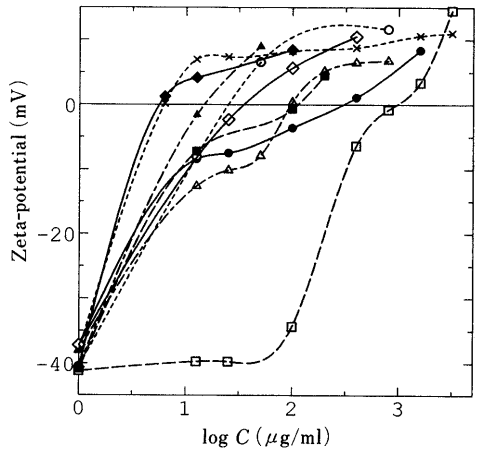


Fig. 1. Relation between Zeta-Potential (mV) and Concentration of Disinfectants (μg/ml)

●, BAC; ○, **4b**; □, **4c**; △, **4d**; ◇, **4e**; ×, **4f**; ■, **5c**; ▲, **5d**; ◆, **5e**.

in the cell, while the increase in ζ-potential again after log 2.8 is that adsorption onto the cell surface occurs due to van der Waals force. Similar phenomena were observed in **4d** and **5c**, but the permeability slightly decreased as compared to that of **4c** for longer alkyl chain groups.

Such phenomena were no longer observed in the compounds with longer alkyl chain groups such as **4e**, **4f**, and **5d**, **5e**. These findings support the idea that adsorption by electrostatic binding predominates with shorter alkyl chain groups, and that adsorption by van der Waals force predominates with longer alkyl chain groups.

We found no change of ζ-potential with **4a**.

Correlation between Disinfectant Concentration at Zero ζ-Potential and *MIC* against *E. coli* The penetration and adsorption of disinfectants into/onto the cell have direct effects on cell growth. We therefore investigated the largest electric change, namely, the electric charge inversion of the cell, to establish the relationship between the disinfectant concentration at $\zeta = 0$ (Fig. 1) and the *MIC* against *E. coli* (Table I). The results are shown in Figs. 2 and 3. With the homologues of **4**, the relation between the logarithm of the concentration of disinfectant (log C) at $\zeta = 0$ and the logarithm of the *MIC* (log *MIC*) was essentially linear with a correlation coefficient of $r = 0.989$ (Fig. 2). The relation between log C and log *MIC* gave a less good correlation ($r = 0.893$) for all of the homologues of **4**, the homologues of **5**, and the BAC type (Fig. 3).

These results support the idea that agents with a high electric charge inversion concentration are powerful antibacterials. In other words, they show strong penetrating

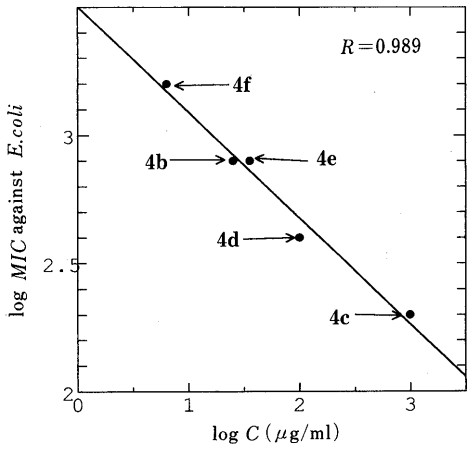


Fig. 2. Relation between log *MIC* and log *C* against *E. coli*

C, the concentration of disinfectant at $\zeta=0$; *MIC*, (μg/ml) against *E. coli*. *R*: correlation coefficient.

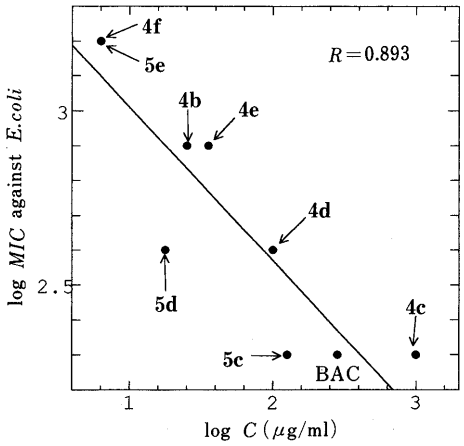


Fig. 3. Relation between log MIC and log *C* against *E. coli*

C, the concentration of disinfectant at $\zeta=0$; *MIC*, (μ/ml) against *E. coli*. *R*: correlation coefficient.

ability into the cell. Agents with a low electric charge inversion concentration are weak antibacterials, and are quickly adsorbed onto the cell surface by van der Waals force, with permeation into the cell being stopped by the electrostatic shielding effect.

Based on the above experimental findings, we suggest that the action mechanism of the disinfectants could be as follows:

Step 1: Penetration of cationic agents into the cell. Step 2: Electrostatic binding and electrical neutralization of the anionic intracellular components in the cell by the cationic surfactants. Step 3: Electrostatic binding of the cationic agents on the cell surface and the continuous adsorption of their hydrophobic groups by van der Waals force onto the cell surface. Step 4: Growth inhibition by ζ-potential inversion.

Experimental

Chemicals The compounds (**4** and **5**) were synthesized by the method described in the previous study.[1] The alkyl groups were octyl (C_8), decyl (C_{10}), dodecyl (C_{12}), tetradecyl (C_{14}), hexadecyl (C_{16}), and octadecyl (C_{18}). BAC (long alkyl chain components, C_{12}: 83% and C_{14}: 17%) was used as a reference material.

Antibacterial Activity Assay *Escherichia coli* obtained from a patient at Shinshu University hospital was used in the antibacterial assay. The *MIC* against *E. coli* was measured by an agar dilution method, using nutrient agar (Nissui) plates.

Preparation of Cell Suspension *E. coli* was cultivated in brain heart infusion broth at 37 °C for 20 h with shaking. After cultivation, *E. coli* was collected by centrifugation (7000 × *g*, 10 min) at 5 °C. The collected cells were washed twice with sterilized physiological salt solution (72 ml of 0.2 M Na_2HPO_4 solution, 28 ml of 0.2 M NaH_2PO_4 solution and 5.0 g of sodium chloride in 1 l) and diluted to a cell concentration of 10^8 cells/ml with a physiological salt solution.

Preparation of Cationic Disinfectant Solutions Each cationic disinfectant was diluted to a concentration of 6.25, 12.5, 25, 50, 100, 200 400, 800, and 1600 μg/ml with a sterilized 1 mM KCl solution.

Measurement of ζ-Potential The ζ-potential was measured by electrophoretic light scattering (ELS-800, Otsuka Electronics). A standard solution contained 0.1 ml of *E. coli* suspension (10^8 cells/ml) in 50 ml of 1 mM KCl solution. The quartz cell used for electrophoresis was washed four times with the standard solution, and filled carefully with standard solution so as not to form bubbles; the ζ-potential was measured and analyzed automatically. In the same manner, just before measurement, a 0.1 ml aliquot of *E. coli* suspension (10^8 cells/ml) was added to 50 ml of cationic disinfectant solution, the concentration of which had been previously adjusted; the quartz cell was washed four times with this solution, after which it was filled carefully with this solution so as not to form air bubbles; the ζ-potential was measured and analyzed automatically.

References

1) M. Makino, S. Ohta, H. Zenda, *Yakugaku Zasshi*, **114**, 73 (1994).
2) M. Makino, S. Ohta, H. Zenda, *Yakugaku Zasshi*, **112**, 712 (1992).
3) S. Ohta, K. Nagai, H. Zenda, M. Makino, *Jpn. J. Hosp. Pharm.*, **18**, 196 (1992).
4) S. Ross, C. E. Kwartler, J. H. Bailey, *J. Colloid Sci.*, **8**, 385 (1953).
5) R. J. Dubos, *J. Exp. Med.*, **86**, 215 (1947).
6) K. McQuillen, *Biochem. Biophys. Acta*, **5**, 463 (1950).
7) B. A. Newton, *J. Appl. Bact.*, **23**, 345 (1960).
8) L. A. Birenbaum, J. G. Nairn, G. C. Walker, *J. Can. Pharm.*, **98**, 378 (1965).
9) K. M. Godfrey, *J. Soc. Cosmetic Chemists*, **17**, 17 (1966).
10) C. Hansch, J. M. Clayton, *J. Pharm. Sci.*, **62**, 1 (1973).
11) T. Uchibori, S. Watanabe, *J. Antibact. Antifung. Agents*, **5**, 13 (1977).
12) T. Uchibori, S. Watanabe, *J. Antibact. Antifung. Agents*, **5**, 527 (1977).
13) R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, B. Vincent, "Microbial Adhesion to Surfaces," Ellis Horwood Ltd., Chichester, 1980.
14) S. Osanai, Y. Abe, *J. Antibact. Antifung. Agents*, **10**, 377 (1982).
15) H. Kourai, F. Machikawa, T. Horie, K. Takeichi, I. Shibasaki, *J. Antibact. Antifung. Agents*, **11**, 401, (1983).
16) H. Kourai, K. Takeichi K. Muramatsu, I. Shibasaki, *J. Antibact. Antifung. Agents*, **17**, 119, (1989).
17) M. Sugiura, A. Yabe, *Nippon Nogeikagaku Kaishi*, **43**, 478 (1969)
18) M. Sugiura, *Kogyo Kagaku Zasshi*, **74**, 4 (1971).
19) D. J. Wilkins, R. H. Ottewill, A. D. Bangham, *J. Theoret. Biol.*, **2**, 165 (1962).
20) E. D. Eylar, M. A. Madoff, O. V. Brody, and J. L. Oncley, *J. Biol. Chem.*, **237**, 1992 (1962).
21) Y. Shigeri, M. Tomizawa, K. Takahashi, M. Koishi, T. Kondo, *Can. J. Chem.*, **49**, 3623 (1971).