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Effect of the Structure of Insoluble Pyridinium-Type Polymer on Its Ability to Capture Bacteria in Water

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Effect of the structure of insoluble pyridinium-type polymer on its ability to capture bacteria in water was investigated. Strong ability of the polymer was further confirmed by an equilibrium capture experiment. The ability was quantitatively evaluated by the removal coefficient reported previously based on initial rate of decrease of viable cell counts caused by the presence of the polymer. The coefficient uniformly increased with the content of pyridinium group, and considerably increased when the content of divinylbenzene became smaller. The coefficient also increased with the content of 4-vinylpyridine in the polymer matrix. Based on these observations, presence of pyridinium group was concluded to be essentially important in the affinity of the polymer for bacterial cells. Hydrophilicity and high degree of swelling in water of the polymer matrix appeared to enhance the ability to capture bacteria.

A recent publication from this laboratory described a novel and remarkable ability of cross-linked poly(4-vinylpyridinium halide) to remove bacteria from water.[1] This insoluble pyridinium-type polymer captured many bacteria alive by contact with them. The polymer was found to capture virus in water strongly with keeping the viral activity.[2] This nature of the polymer appears to be of wide application in microbiology and biotechnology.

We are now attempting to immobilize microbial cells on this polymer in order to apply the new technology to the production of useful materials by biochemical reactions. Although various methods are known for the immobilization of enzymes and microbial cells, continuous efforts are being made to pursue new materials and techniques for the immobilization.[3] Immobilization of these biocatalysts to solid materials by chemical reaction is apt to result in damage to the enzyme activity. Entrapment of them in the gel of polymeric substances is apt to result in a decrease of the contact of substrates with enzymes and microbial cells. Adsorption onto ion-exchange resin is not strong and is reversible. However, immobilization on the insoluble pyridinium-type polymer can be realized without chemical reaction and entrapment. Surface-immobilized, live bacteria entities on this polymer are expected to show useful properties.

Prior to this research work, it is necessary to obtain information about basic properties of the insoluble pyridinium-type polymer. As was reported previously,[1] the capturing ability of the polymer is not limited to viable cells. The difference in the ability for viable and killed bacteria was insignificant, and physicochemical interaction appeared to be much more important than physiological action in the phenomenon. In this work, we have investigated effect of the structure of the polymer on its ability to capture bacteria.

Experimental

Materials. 4-Vinylpyridine provided by Koei Chemical Co. Ltd., Osaka, and commercial products of divinylbenzene,[5] styrene, ethanol and toluene were purified by distillation before polymerization. Commercial products of 2,2′-azobisisobutyronitrile, benzyl bromide, and other chemicals were used without further purification.

Insoluble Pyridinium-Type Polymer. Cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide) was used as the insoluble pyridinium-type polymer. Procedure for the preparation of the polymer is given below. Polymerizations were carried out in a 2-l round-bottomed three-necked flask under a nitrogen atmosphere. Copolymerization of 4-vinylpyridine with divinylbenzene, and that of 4-vinylpyridine with styrene and divinylbenzene were carried out using 2,2′-azobisisobutyronitrile as initiator at 75 °C for 5 to 6 h according to the procedure of Katchalsky et al.[4] with minor modifications.[5]

The copolymer was converted to the insoluble pyridinium-type polymer as follows. The copolymer was added to methanol, and the mixture was kept at room temperature for about 20 h. Methanol was separated by filtration. The swollen copolymer was added to toluene in a three-necked flask connected with a mechanical stirrer and a reflux condenser. The mixture was stirred gently, and the prescribed amount of benzyl bromide was added: The solution was allowed to react at 60 to 80 °C for 3 h under stirring. After the reaction, the polymer was separated by filtration, and was washed by benzene, methanol and water followed by drying in vacuo to constant weight. In order to determine content of pyridinium group, the polymer in the bromide form was transferred into the polymer in the nitrate form by treatment with excess 1 M† potassium nitrate in a column method. Titration of the eluted solution with standard 0.1 M silver nitrate using eosin as indicator showed the content of pyridinium group in the polymer.

For comparison with the insoluble pyridinium-type polymer, four commercial resins were used in this work. Amberlite IRA-900 and IRA-400 supplied by Rohm & Haas Co., Philadelphia, Pa., were used as strong base anion-exchange resins, which had a styrene-divinylbenzene matrix with quaternary ammonium group. The resins were preconditioned according to a usual method,[5] and were

† 1M=1 mol dm^{-3}.

used in the chloride form. Amberlite IRA-45 supplied by Rohm & Haas Co. was used as a weak base anion-exchange resin, which had a styrene-divinylbenzene matrix with primary, secondary, and tertiary amino groups. The resin was preconditioned to the free base form in a similar manner. Amberlite XAD-4 supplied by Rohm & Haas Co. was used as porous styrene-divinylbenzene resin with no ion-exchange functional group. This nonionic resin was preconditioned by washing with methanol followed by washing with water.

Bacteria. *Escherichia coli* was incubated and treated as was reported previously.[1]

Procedure. All procedures of the removal experiments were carried out under aseptic conditions as was reported previously.[1]

Serial dilutions of cell suspensions were done of the medium prepared by dissolving KH_2PO_4 (4.5 g), Na_2HPO_4 (6.0 g), L-cystin·HCl (0.5 g), and tween 80 (0.5 g) in 1000 ml of water. The diluted cell suspensions were transferred to plates of Eosin-Methylene Blue-agar (BBL). The measurements of viable cell counts were done after incubation at 37 °C for 24 h.

Results and Discussion

Ability of the insoluble pyridinium-type polymer to capture bacteria was evaluated by the removal coefficient defined as:[1]

$$\text{Removal coefficient} = \frac{V}{Wt}\log\,[N(0)/N(t)]$$

Here, $N(0)$ is the initial viable cell count, $N(t)$ is the viable cell count at contact time t, V is the volume of viable cell suspension, W is the dry weight of the polymer, and t is the contact time. The coefficient was shown to be independent on initial cell count $N(0)$ and concentration of insoluble polymer W/V.[1] Although the difference in the content of pyridinium group should be taken into consideration, cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide and chloride) were most effective among the polymers examined. Although commercial resins also showed the ability to some extent, that of the insoluble pyridinium-type polymer was outstanding.

The removal coefficient is a dynamic index of the ability of polymers to capture bacteria, and is based on initial rate of decrease of viable cell counts. On the other hand, equilibrium capture experiment would also be important for the evaluation of the basic and definitive ability of the polymer to capture bacteria.[6] Thus we have performed the equilibrium capture experiment at 37 °C. After the mixture of polymer and *E. coli* in sterilized physiological saline reached equilibrium, viable cell counts were determined. The experimental isotherm for *E. coli* on the insoluble pyridinium-type polymer is shown in Fig. 1 (graph A). The isotherm on cross-linked poly(4-vinylpyridine), i.e., the polymer without pyridinium group is shown in Fig. 1 (graph D). The isotherms on various other polymers are also shown in Fig. 1 (graphs B, C, and E). As can be seen in Fig. 1, the insoluble pyridinium-type polymer was confirmed to have a much stronger ability to capture bacteria when compared with that of other types of polymers examined.

The ability of the insoluble pyridinium-type polymer to capture bacteria appeared to depend on the content of pyridinium group.[1] We have examined the effect using cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide) containing various amount of the pyridinium group. In Fig. 2 is shown the result. The coefficient uniformly increased with the content of pyridinium group. This result indicates the importance of the presence of pyridinium group in the ability of the polymer to capture bacteria on its surface. Electrostatic interaction between the positive charge on the surface of the polymer and the negative charge of the microbial cell surface appears to be

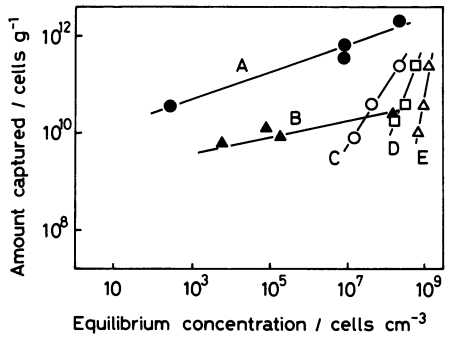

Fig. 1. Experimental isotherms of the capture of *E. coli* by insoluble polymers. (A): Cross-linked poly(N-benzyl-4-vinylpyridinium bromide) containing 72 mol% of 4-vinylpyridine and 2.9 mmol g^{-1} of the pyridinium group, (B): Amberlite IRA-45 in the free base form, (C): Amberlite XAD-4, (D): Cross-linked poly(4-vinylpyridine) containing 72 mol% 4-vinylpyridine, (E): Amberlite IRA-400 in the chloride from.

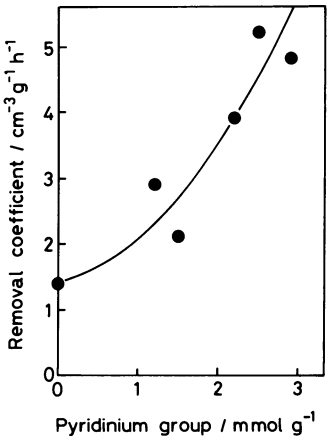

Fig. 2. Effect of the content of pyridinium group in the insoluble pyridinium-type polymer on the removal coefficient for *E. coli*. The polymer contained 72 mol% of 4-vinylpyridine.

Table 1. Effect of the Structure of Cross-Linked Poly(*N*-benzyl-4-vinylpyridinium Bromide) on the Removal Coefficient for *Escherichia Coli*

Run	Monomer content/mol%			Pyridinium group	$N(0)$	W[d]	Removal coefficient
	4-VP[a]	DVB[b]	ST[c]	mmol g^{-1}	cells cm^{-3}	dry g	$cm^3\ g^{-1}\ h^{-1}$
1	72	28	0	2.9	4.3×10^6	4.0	4.8
2	72	20	8	2.9	6.9×10^7	4.4	6.9
3	72	10	18	2.9	1.5×10^7	5.8	7.1
4	72	3	25	3.1	1.5×10^7	4.5	∞[e]
5	72	28	0	2.2	6.9×10^7	3.5	3.9
6	54	28	18	2.1	4.1×10^7	2.7	2.7
7	36	28	36	2.1	4.1×10^7	3.2	1.6

a) 4-Vinylpyridine. b) Divinylbenzene. c) Styrene. d) The volume of bacterial suspension, V, was 20 ml. e) Viable cells were not detected even at the contact time of 0.5 h.

important, although the strong effect of the benzyl group is not clear.

Cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide) was prepared under various conditions in order to examine the effect of higher structure of the polymer upon its ability to capture bacteria. Results are given in Table 1.

Runs 1 to 4 were performed to examine the effect of the degree of cross-linking of the polymer. Here, content of 4-vinylpyridine and that of the pyridinium group were kept nearly constant, and part of divinylbenzene was replaced by styrene. As is obvious in Table 1, the removal coefficient increased with the decrease of the content of divinylbenzene. The result shows that decrease of the degree of cross-linking caused the increase of the ability of the polymer to capture bacteria. In this case, the polymer swells very well in water. The effective surface area in water is much larger, which enhances the ability of the polymer to capture bacterial cells.

Runs 5 to 7 were carried out to examine effect of composition of the matrix of the polymer. In these cases, content of pyridinium group was kept nearly constant at 2.1 mmol g^{-1}, and that of divinylbenzene was also fixed around 28%. As can be seen in Table 1, the removal coefficient increased with the content of 4-vinylpyridine. This result shows that hydrophilicity of the polymer matrix enhanced the affinity of the polymer for bacterial cells.

It would be noteworthy to remind that cross-linked poly(*N*-alkly-4-vinylpyridinium bromides) were much less effective than cross-linked poly(*N*-benzyl-4-vinylpyridinium bromide) for the capture of bacterial cells.[1] Hydrophobicity of the long alkyl group of the polymer did not strengthen the ability to capture bacteria. The *N*-dodecyl polymer and the *N*-hexadecyl polymer showed smaller values of the removal coefficient than that of the polymer with no pyridinium group, i.e., cross-linked poly(4-vinylpyridine). Hydrophobicity of the polymer seems to diminish its ability to capture bacteria.

The ability of the insoluble pyridinium-type polymer to capture bacteria is much higher than that of tetraalkylammonium-type anion-exchange resins. With respect to the strength of positive charge, tetraalkylammonium-type resin would not be inferior to the insolube pyridinium-type polymer. The difference between the two types of polymers in the ability to capture bacteria seems to be ascribable mainly to the difference in hydrophilicity. The pyridinium group is highly hydrophilic. For example, linear polymer of poly(*N*-benzyl-4-vinylpyridinium bromide) is hygroscopic.

References

1) N. Kawabata, T. Hayashi, and T. Matsumoto, *Appl. Environ. Microbiol.*, **46**, 203 (1983).

2) N. Kawabata, T. Hashizume, and T. Matsumoto, *Agric. Biol. Chem.*, **50**, 1551 (1986).

3) S. Fukui and A. Tanaka, *Ann. Rev. Microbiol.*, **36**, 145 (1982).

4) A. Katchalsky, K. Rosenheck, and A. Altman, *J. Polymer Sci.*, **23**, 955 (1957).

5) N. Kawabata and K. Ohira, *Environ. Sci. Technol.*, **13**, 1396 (1979).

6) K. DeNicola and D. J. Kirwan, *Biotechnol. Bioeng.*, **22**, 1283 (1980).