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Adsorption of Pb^{2+} in the Components of Bacterial Cell Membrane

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***S. epidermidis* cell was fractionated into cell wall, cell membrane and cytoplasm. The cell membrane adsorbed the most abundant Pb^{2+} per unit dry weight of the three fractions tested. Adsorption behavior of Pb^{2+} in lipid and protein, which are the main components of the cell membrane, indicated that phosphatidylethanolamine and phosphatidylinositol having phosphoryl group and gangliosides containing carboxyl groups adsorbed much more Pb^{2+} than triglycerides lacking any chargeable functional groups. Protein purified from cell membrane adsorbed larger amount of Pb^{2+} than total native cell membrane or cell membrane lipid.**

Key words: ***Staphylococcus epidermidis*, lead ion, adsorption, cell membrane, lipid, protein**

Heavy metals dissolved in soil, waste water, sea water and activated sludge filter into living organisms through the food chain, accumulating in the organisms with little excretion. Human being is situated on the top of the food chain, and thus exposed to the dangerous toxications due to incrassation of heavy metals in the body.

Microorganisms can be harvested abundantly at a low price. Separation of heavy metals from aqueous solutions using microorganisms is more economic and efficient than the conventional chemical method of separation because the adsorption and desorption of heavy metals by microorganisms proceed faster than by the chemical method. Selective separation of heavy metals can be done easily and disposal of the wasted microorganisms poses few problems. Adsorption mechanism of heavy metals consists of absorption, precipitation, complex formation, ion exchange, active transport and so on (25). Adsorption of heavy metal ions in the microorganisms is mainly carried out through precipitation in the cell and through interactions between heavy metal ions and functional groups existing in the cell wall or on the surface of the cell membrane (24). Adsorption of heavy metal ions is affected by environmental conditions such as temperature, pH, cell mass and presence of other ions (5, 15). There exist various kinds of functional groups on the cell surface, which has a great influence on the adsorption kinetics of the positively charged metal ions (4). Binding sites for the heavy metal ions are negatively charged functional groups such as carboxyl, thiol and phosphate groups. Amine group can also play the role as a binding site for the heavy metal ions through coordination on to the unpaired electrons. Biopolymers, especially proteins, having charged functional groups, can bind selectively to Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+} and Ca^{2+} by way of side chains of metal-binding proteins (26). Precipitating locations and binding sites of heavy metal ions in the cell have been subjects of great number of researches to effectively dispose of the waste heavy metal ions by microbiological means. Nakajima *et al.* (20) and Feofilova *et al.* (9) examined main constituents of cells binding to heavy metal ions through observations of heavy metal ion adsorption onto cell components chemically pretreated. Doyle *et al.* (8) disclosed principal functional groups binding to heavy metal ions by modifying the charge of the various functional groups present in the cell components.

In this study, cell membrane was separated from fractionated *Staphylococcus epidermidis* cells. We attempted to locate the major binding sites by comparing the metal ion uptake values of protein with that of lipid extracted from the cell membrane.

Materials and Method

Strain and culture conditions

Staphylococcus epidermidis EVANS ATCC 12228 obtained from the National Health Research Center was cultivated according to the methods previously reported (16).

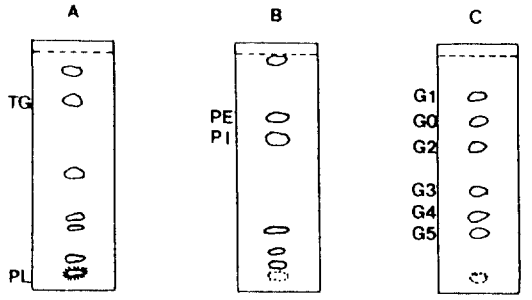

Fig. 1. Separation of lipids by in *S. epidermidis* TLC. Dot Line is spotting point. A: heptane (60); diethylether (40); glacial acetic acid (20), B: chloroform (60); methanol (50); glacial acetic acid (1); water (4), C: propanol (7); water (3), TG: triglycerides, PL: phospholipids, PE: phosphatidylethanolamine, PI: phosphatidylinositol, G 0-G5: gangliosides.

Separation of cell membrane

Cell walls were separated first from *S. epidermidis* cells as described by Doyle and Birdsell (7). The resulting supernatant was ultracentrifuged (100,000×*g*, 40 min, 4℃, Beckman XL-90) to obtain cell membrane and cytoplasm as precipitate and supernatant respectively. The cell membrane thus harvested was confirmed by following the method proposed by Schnaitman (22). Dry weight of the cell membrane was measured after drying the suspension at 105℃ for 18 hours.

Preparation of cell membrane

Lipids were separated from cell membrane by the method reported by Beaven *et al.* (1) with some modifications. 0.5 ml chloroform was added to the dried cell membrane to develop the lipids on a TLC plate. Developing solvent for triglyceride (TG) and phospholipid (PL) was a mixture of heptane, diethylether and glacial acetic acid (60 : 40 : 20 (v/v)), while phosphatidylethanolamine (PE) and phosphatidylinositol (PI) were developed using a mixture of chloroform, methanol, glacial acetic acid and water (60 : 50 : 1 : 4 (v/v)). Gangliosides (G) was developed with propanol-water mixture (7 : 3 (v/v)). Colorization by iodine vapour (12) on the developed TLC plate after drying at room temperature enabled us to localize the lipids (Fig. 1). Lipid extracted from each band by chloroform was dried at 50℃ until constant weight was attained.

Preparation of proteins of cell membrane

Proteins were extracted from cell membrane by Mizuno and Kageyama's method (19) with some modifications. The harvested cell membrane was suspended in 0.1 M citrate-phosphate buffer (pH 7.0) containing 0.03 M 2-mercaptoethanol, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.5% lysozyme and 0.5% Triton X-100. The suspension solution was warmed to 30℃ for a few minutes, kept at room temperature for 60 minutes, and then incubated at 37℃ for 2 hours. The proteins of cell membrane containing Triton X-100 and EDTA were quantified using UV/VIS Spectrophotometer (Shimadzu, UV-120-02). To remove the EDTA remaining in the proteins, the proteins were dialyzed three times at intervals of 2~3 hours with 50 mM Tris-HCl buffer solution (pH 7.5) at 4℃. Triton X-100 was eliminated from the dialysis solution by ion exchange chromatography using DEAE-sepharose gel. The proteins lacking EDTA and Triton X-100 were analyzed quantitatively according to the Lowry *et al.*'s method (18). The proteins of cell membrane thus obtained were subjected to Pb^{2+} adsorption experiments after concentration with saturated ammonium sulfate solution followed by dialyzing for 24 hours at 4℃ twice with sterilized and deionized water to remove the residual metal ions of the buffer solution.

Adsorption of Pb^{2+}

When cell membrane, lipids and proteins from cell membrane were employed for the metal ion uptake, 4.8 ml of sterilized and deionized water was added to 50 ml flask together with 10^{-3} M Pb^{2+} solution ($Pb(NO_3)_2$), and then solution pH was adjusted to 6.0. Pb^{2+} adsorption was performed for 1 hour at room temperature with 0.1 ml (dry weight 2 mg) adsorbent suspension. In case of whole cell, 48 ml of sterilized and deionized water, 1 ml of 10^{-4} M Pb^{2+} solution (pH 6.0) and 1 ml of cell suspension (dry weight 20 mg) were used for the Pb^{2+} uptake experiments. Whole cells and cell walls were centrifuged at 12,000×*g* for 20 minutes and at 39,000×*g* for 30 minutes respectively at the end of adsorption. Recovery of cell membrane from the Pb^{2+} solution was done by an ultracentrifuge (100,000×*g*, 40 min, 4℃), while lipids and proteins were separated by dialysis using sterilized and deionized water for 3 days. Amounts of the adsorbed Pb^{2+} in the adsorbents was determined by comparing concentration of Pb^{2+} dissolved in the solution before and after the adsorption, using Atomic Absorption Spectrophotometer (Shimadzu, AA-670).

Results and Discussion

Pb^{2+} adsorption according to the culture time

Content of the cell constituents changes with phase of the cell growth (21). Fig. 2 shows the Pb^{2+} uptake by whole cell as a function of culture time. It can be seen that *S. epidermidis* at exponential growth phase adsorbed Pb^{2+} at a rate similar to that at stationary phase. Horikoski *et al.* (13) found UO_2^{2+} uptake by *Streptomyces viridochromogens* was nearly independent of the culture time. On the contrary, Friis and Myers-Keith (10) obser-

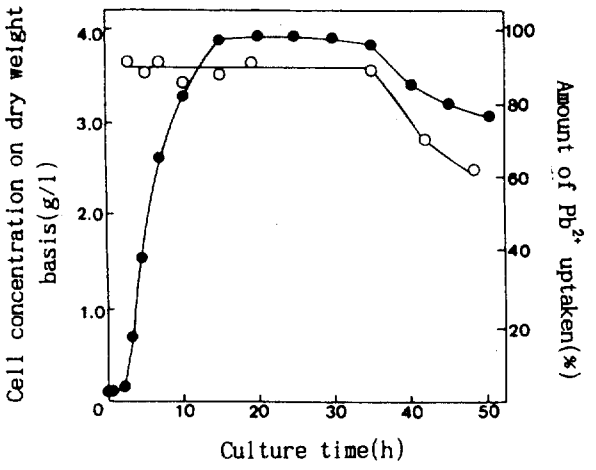

Fig. 2. Effect of culture time on the uptake of Pb^{2+} by *S. epidermidis*. ●: growth curve in nutrient broth, ○: amount of Pb^{2+} uptaken.

Table 1. Pb^{2+} uptake capacity of *S. epidermidis* grown in different growth media.

Growth media	Cell mass grown for 7 h of incubation (g/l)	Amount of Pb^{2+} uptaken (%)
Nutrient broth	0.416	88
7.5% NaCl containing nutrient broth	0.012	87

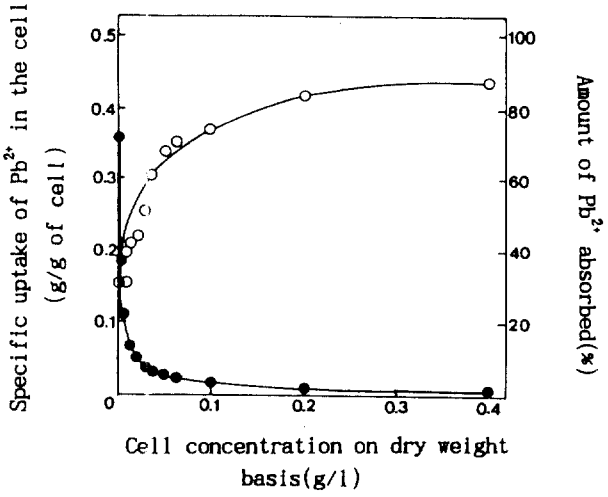

Fig. 3. Uptake of Pb^{2+} with increase in cell concentration by *S. epidermidis*. ●: specific uptake of Pb^{2+} in the cell, ○: amount of Pb^{2+} uptaken.

Table 2. Composition of cell membrane in *S. epidermidis* cell.

Component	Approximate composition (%)
Protein	62
Lipid	26
Others	12

ved reduced rate of UO_2^{2+} uptake by *Streptomyces longwoodensis* at lag phase and enhanced uptake rate at exponential phase of growth. They ascribed the enhanced rate of the metal ion uptake to the increase in phosphorus content of the cell. Living microorganisms (23), dead cells (6) or freeze dried cells (3) have been employed for the metal uptake. Fig. 2 exhibits a declined uptake rate at death phase to conclude the Pb^{2+} uptake by *S. epidermidis* depended on metabolism of the living cells to some extent. The composition of the culture medium can also affect the content of the cell constituents. Cell walls of *Staphylococcus aureus* H cultivated in nutrient broth (NB) containing 7.8% NaCl became more enriched with teichoic acid and adsorbed larger amount of Mg^{2+} than those cells grown in NB free of NaCl (11). 7.5% NaCl in the growth medium repressed the cell growth rate of *S. epidermidis*, but didn't give any influence on the Pb^{2+} uptake behavior (Table 1).

Fig. 3 describes the total amount of Pb^{2+} adsorbed increases while the specific amount of Pb^{2+} adsorbed per unit weight of adsorbent decreases with cell mass involved in the uptake experiments. This should be due to equilibrium shift resulting from reduced concentration of Pb^{2+} remaining dissolved in the solution as the mass of the adsorbent increased.

Pb^{2+} adsorption by lipids of cell membrane

S. epidermidis cells were composed of 15% cell walls, 8% cell membrane and 56% cytoplasm (on dry weight basis). The cell membrane adsorbed the largest amount of Pb^{2+} of the three adsorbents tested (16). Composition of cell membrane of *S. epidermidis* was collected in Table 2.

Main constituents of lipids of cell membrane were Triglycerides (TG), Phospholipids (PL) and Sphingolipids (SPL). The PL consisted of Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), Phosphatidylserine (PS) and Phosphatidylinositol (PI), while the SPL was composed of Sphingomyelin (SPH), Cerebrosides (CE) and Gangliosides (G) (17). Lipids from *S. epidermidis* cell membrane was developed on a TLC plate as shown in Fig. 1. The presence of TG was confirmed by Higgin's method (11). PL was separated into PE and PI, while SPL was composed of different kinds of G's (Fig. 1). As depicted in Fig. 4, the specific uptake of Pb^{2+} per unit weight was 0.314, 0.328, 0.328, 0.300 and 0.136 for total lipids, PE, PI, G and TG respectively. PE and PI molecules have a negatively charged phosphoryl group at the end of the molecules, to which Pb^{2+} ion was expected to be bound. The carboxyl group existing in N-acetylneuraminate was supposed to be responsible for the Pb^{2+} binding in G. However it is to be noted that significantly

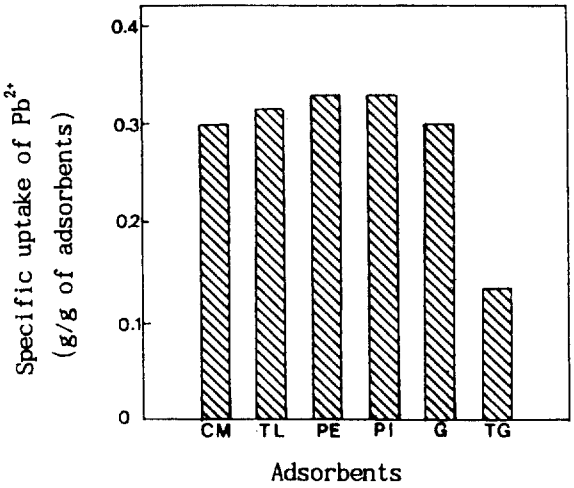

Fig. 4. Adsorption of Pb^{2+} by lipids of cell membrane in *S. epidermidis*. CM: cell membrane, TL: total lipids of cell membrane, PE: phosphatidylethanolamine of cell membrane, PI: phosphatidylinositol of cell membrane, G: gangliosides of cell membrane, TG: triglycerides of cell membrane.

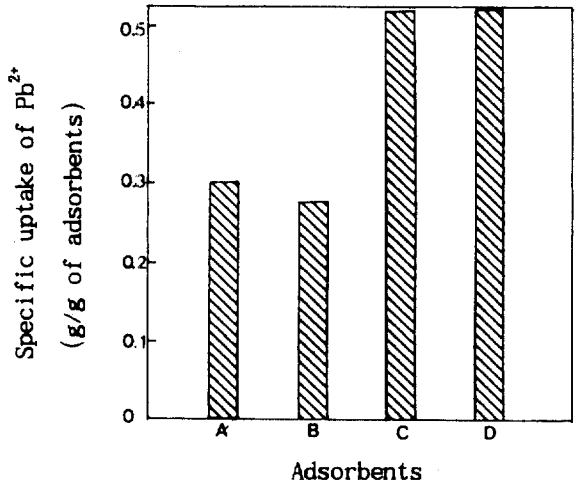

Fig. 5. Adsorption of Pb^{2+} by protein of cell membrane in *S. epidermidis*. A: cell membrane, B: cell membrane protein containing EDTA and Triton X-100, C: cell membrane protein free from EDTA, D: cell membrane protein free from EDTA and Triton X-100.

reduced amount of Pb^{2+} was adsorbed in TG lacking any chargeable functional groups.

From these points of view, it can be said that Pb^{2+} uptake by lipids of cell membrane occurred by way of interactions between Pb^{2+} and negatively charged functional groups in the adsorbents. Beveridge and Murray (2) suggested Pb dissolved in aqueous solution could exist not only as Pb^{2+} but also in a form of anionic complexes such as $Pb(OH)_3^-$ and $Pb(OH)_4^{2-}$. Therefore electropositive amine groups could be potent chelators for the negatively charged ions. However both PI having only phosphoryl groups and PE containing amine groups as well as phosphoryl groups adsorbed almost same amount of Pb^{2+} (Fig. 4). Hence few Pb^{2+} ions can be said to bind to amine groups in PI. Hydroxyl groups in PI and G are polar but uncharged at neutral pH. The fact that adsorption of Pb^{2+} in PI having hydroxyl groups was found to be similar to that in PE lacking hydroxyl groups (Fig. 4) lead us to conclude that the Pb^{2+} uptake depend negligibly on the presence of hydroxyl groups. Tobin *et al.* (24) reported equivalent experimental results.

Pb^{2+} being a bivalent cation is expected to bind to two phosphoryl groups in PE and PI. Two carboxyl groups of N-acetylneuraminate in G would also bind to Pb^{2+} as each Ca^{2+} ion was bound to two carboxyl groups of α-D-galactopyranosiduronate (14). Rate of Pb^{2+} uptake based on unit weight of adsorbent not only in PE but also in PI was higher than that corresponding to G. However molecules per unit mass of PE and PI are about two times as numerous as those of G, implying number of carboxyl groups of G is two times as low as that of phosphoryl groups of PE and PI. Hence carboxyl groups of G can be said to be more capable of Pb^{2+} uptake than phosphoryl groups of PE and PI.

Pb^{2+} adsorption by proteins of cell membrane

Fig. 5 shows Pb^{2+} adsorption capacity of cell membrane proteins is higher than that of cell membrane lipids. Proteins containing EDTA and Triton X-100 adsorbed only 0.275 g of Pb^{2+} per g of adsorbents. On the contrary, cell membrane proteins free from EDTA exhibited 0.497 g of Pb^{2+} uptake. When both EDTA and Triton X-100 were extracted from the cell membrane proteins, adsorption of Pb^{2+} was achieved up to 0.499 g/g of adsorbent. EDTA being a strong desorbent of metal ions should have inhibited the binding of Pb^{2+} to functional groups of the proteins. Effect of residual Triton X-100 was proved to be insignificant compared with that of EDTA. On the other hand, Yu *et al.* (27) reported Triton X-100 increased accumulation of Ca^{2+} in *Hansenula anomala* up to 40%.

Cell membrane of *S. epidermidis* was composed of 62% proteins and 26% lipids as presented in Table 2. Pb^{2+} uptake in cell membrane was lower than that in cell membrane lipid (Fig. 4) contrary to expectation. This could be ascribed to the fact that functional groups of fractionated cell membrane lipids and cell membrane proteins were more easily exposed to Pb^{2+} ion than those of cell membrane where the proteins located within the lipid layer were surrounded tightly by lipid molecules.

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