

Removal of Adherent Bacteria from Catheter Materials *in vitro* by *N*-Acyated Amino Acids

Kazushi SEO¹⁾, Hiroshi NAKANO²⁾, Tsuguru USUI¹⁾,
Yoichiro MIYAKE³⁾ and Hidekazu SUGINAKA³⁾

1) Department of Urology, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

2) Department of Urology, Mazda Hospital, 2-5, Aosakiminami Fuchu-cho, Aki-gun 735, Japan

3) Department of Microbiology, Hiroshima University School of Dentistry, Hiroshima 734, Japan

ABSTRACT

Indwelling urinary catheters may act as a reservoir of bacteria and cause urinary tract infections. Removal of the bacteria adherent to a urinary catheter should reduce the incidence of catheter-associated urinary tract infections. Using several *N*-acylated amino acids with combinations of four different acyl residues, we investigated their efficacy in removing adherent bacteria from catheter materials *in vitro*. Among them, acyl phenylalanine and acyl glycine (with acyl lengths of eight or ten) exhibited the highest ability to remove adherent bacteria.

Key words: *N*-acylated amino acid, Bacterial adhesion, Catheter material, Urinary tract infection

Urinary tract infections associated with indwelling urinary catheters are the most common hospital-acquired infections, constituting more than 30% of all nosocomial infections⁷⁾. It is well known that prolonged use of indwelling urinary catheters induces bacteriuria and urinary tract infections. Experiments using an animal model have demonstrated that the use of urinary catheters can produce urinary tract infections¹⁾. Several routes by which bacteria are introduced into the urinary bladder have been suggested⁹⁾, and it is thought that the adherence of bacteria to urinary catheters probably play an important role in the initiation of urinary tract infections. The adherence of bacteria to catheters has been studied. Sugarman reported on the *in vitro* adherence of *Escherichia coli* and *Klebsiella pneumoniae* to catheters¹⁵⁾.

For the prevention of urinary tract infections associated with indwelling catheters, systemic antibiotic prophylaxis and urinary bladder irrigation by antibiotics or disinfectants are generally used. However, these methods are not effective in preventing urinary tract infections of patients with prolonged catheterization. Moreover, the prolonged use of antibiotics causes the emergence of resistant bacteria, and disinfectants have toxic effects on mucosa surfaces.

In the present study, we investigated the *in vitro* ability of *N*-acylated amino acids to remove adherent bacteria (*Pseudomonas aeruginosa* and *Serratia marcescens*) from catheter materials.

P. aeruginosa and *S. marcescens* were isolated at the Department of Urology, Hiroshima University Hospital from 1978 to 1984 and identified using the Abbott Quantum II-BID System (Abbott Laboratories, Irving, TX, U.S.A.) and Oxi/ferm Tube II (Roche Japan, Tokyo, Japan) at the Department of Bacteriology of Hiroshima University Hospital. The bacteria were grown in trypto-soy broth at 37°C for 24 h, harvested by centrifugation, washed in 0.01 M phosphate-buffered saline (PBS, pH 7.0), and then resuspended in PBS at a concentration of 1.0×10^7 /ml.

N-Acyated amino acids.

N-Acyated amino acids were synthesized by reacting a fatty acid chloride and an amino acid under alkaline conditions. Combinations of four different acyls (C4, C6, C8, C10) and four amino acids (glycine, phenylalanine, asparaginic acid, glutamic acid) were used (Fig. 1). *N*-Acyated amino acids were dissolved in PBS at a concentration of 0.01 M and the pH was adjusted to 7.0.



n : 4, 6, 8, 10

R : H, CH₂C₆H₅, CH₂COOH



Fig. 1. Chemical formula of the *N*-acyated amino acids.

MATERIALS AND METHODS

Bacteria.

Catheter materials.

Silicon rubber (Created Medic Co. Ltd., Yokohama, Japan) and latex rubber (Unitika Co., Kyoto, Japan) in 1-mm sheets were used.

In vitro adherence assay.

The catheter material sheets were cut into small pieces (10 × 10 mm). Four pieces were placed in a plastic Petri dish (15 × 90 mm, Corning Glass Works, Corning, NY), to which 50 ml of bacterial suspension was added. Petri dishes were incubated at 37°C from 15 min to 2 h. The rubber pieces were then removed from the Petri dishes, washed with PBS, and fixed in 2.5% glutaraldehyde in PBS. The number of adherent bacteria in ten high power fields were counted by scanning electron microscopy (× 1,000 magnification) for each rubber piece. The mean number of adherent bacteria and the standard deviation per millimeter square were subsequently calculated for the 4 rubber pieces from each Petri dish.

Removal of adherent bacteria.

Catheter material pieces with adherent bacteria were incubated in several *N*-acylated amino acids solutions at 37°C for 1 h. PBS was used as the control. The mean number of remaining bacteria was determined as described above.

Treatment of catheter from patient with *N*-acylated amino acid.

An almost completely obstructed catheter was obtained from a patient with a nephrostomy. The catheter was cut into pieces, which were almost same condition and washed at room temperature for 15 min in octyl phenylalanine solution or PBS as a control. The samples were then observed by scanning electron microscopy.

Scanning electron microscopy.

Specimens were fixed in 2.5% glutaraldehyde in PBS for 16 h and dehydrated in a series of graded ethanol solutions (50-100%). The specimens were dried *in vacuo* for 1 week, and then coated with platinum up to 60 Å thick. Specimens were exa-

mined with a scanning electron microscope (JSM, T-200, JEOL, Tokyo, Japan), and photographed.

RESULTS

The *in vitro* adherence of *P. aeruginosa* and *S. marcescens* to catheter materials is shown in Fig. 2. Both strains were more adherent to latex rubber pieces than to silicon pieces, and the number of adherent cells increased with time. The adherence of *P. aeruginosa* to both materials was significantly greater than that of *S. marcescens*.

Octyl phenylalanine was tested for its ability to remove adherent *P. aeruginosa* and *S. marcescens* from silicon rubber pieces *in vitro* (Table 1). The removal rate of *P. aeruginosa* was 69%, and that of *S. marcescens* was 91%.

We determined the effect of changes in acyl length and amino acid substitutions on the ability of *N*-acylated amino acids to remove *P. aeruginosa* from silicon pieces. Acyl glycines with acyl residues of eight and ten carbons exhibited the highest ability to remove bacterial cells (Fig. 3). Octyl amino acids were also synthesized using four different amino acids, and their ability to remove adherent bacteria was assessed. Octyl glycine and octyl phenylalanine were found to be superior to octyl asparaginic acid and octyl glutamic acid (Fig. 4).

The efficacy of octyl phenylalanine for cleansing

Table 1. The ability of octyl phenylalanine to remove adherent *P. aeruginosa* and *S. marcescens* from silicon *in vitro*.

Bacterium	Mean adherent cell number/mm ² ± S.D.	
	no treatment	treatment
<i>P. aeruginosa</i>	5,983 ± 934	1,828 ± 98 (69)*
<i>S. marcescens</i>	391 ± 38	34 ± 8 (91)

* The numbers in parenthesis represent the percentage of bacteria removed by octyl phenylalanine treatment.

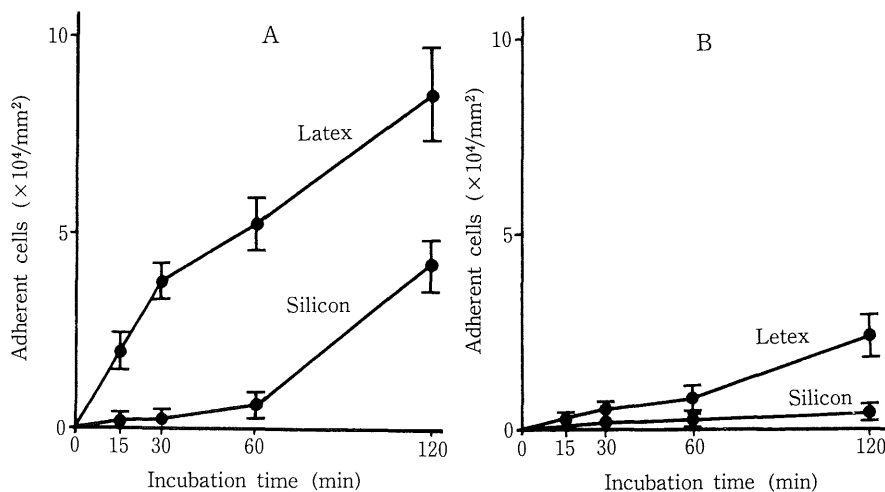


Fig. 2. Time course of bacterial adherence to catheter materials. (A) *P. aeruginosa*. (B) *S. marcescens*.

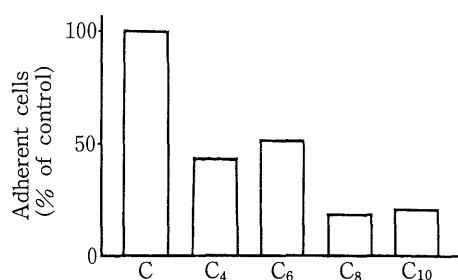


Fig. 3. Effect of acyl length on the efficacy of acyl glycine in removing adherent *P. aeruginosa*. (C) control. (C₄ - C₁₀) acyl glycine.

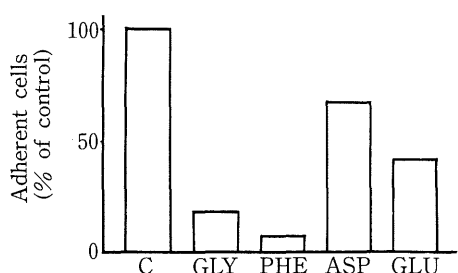


Fig. 4. Efficacy of different octyl amino acids in removing adherent *P. aeruginosa*. (C) control. (GLY) acyl glycine. (PHE) acyl phenylalanine. (ASP) acyl asparaginic acid. (GLU) acyl glutamic acid.

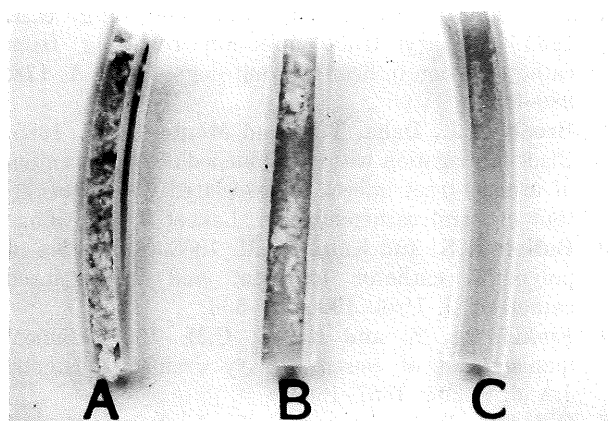


Fig. 5. The efficacy of octyl phenylalanine in removing the biofilm from a completely obstructed catheter obtained from a patient. (A) before treatment. (B) after washing with PBS. (C) after washing with octyl phenylalanine.

catheters was studied *in vitro* using an almost completely obstructed catheter obtained from a patient. Macroscopic observation showed that washing with PBS could not remove the deposits on the inner surface of the catheter, possibly a biofilm, while treatment with octyl phenylalanine did almost remove this biofilm (Fig. 5).

The inner surface of the catheter from the pa-

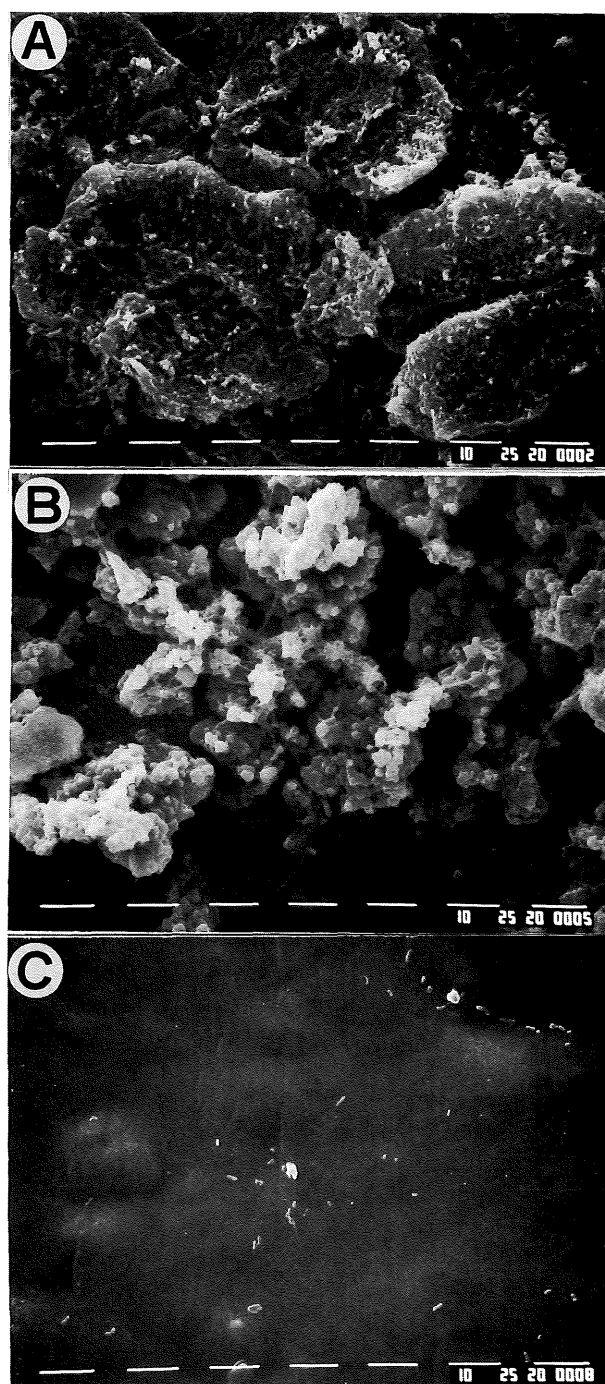


Fig. 6. Scanning electron micrograph of a catheter. (A) completely obstructed catheter obtained from a patient. (B) catheter washed with PBS. (C) catheter washed with octyl phenylalanine. Bar indicates 10 μ m.

tient was also observed by scanning electron microscopy. It was found to be covered with a thick biofilm composed of urinary crystalline constituents and bacterial cells, which remained even after washing with PBS. In contrast, after washing with octyl phenylalanine the biofilm was almost completely removed and only scattered bacterial cells were observed (Fig. 6).

DISCUSSION

The adherence of microorganisms to the urinary catheter surface may have a role in the initiation of urinary tract infections associated with urinary catheters. Other infections have been likewise associated with medical apparatus¹²⁾. For prevention of urinary tract infections associated with urinary catheters, the inhibition of bacterial adherence to a catheter surface or the removal of adherent bacteria may be effective.

Systemic antibiotic prophylaxis and the use of closed sterile drainage systems has greatly decreased the overall incidence of catheter-associated urinary tract infections^{4,5)}. With these aims in mind, various catheter materials have been developed, such as antibiotic-impregnated⁹⁾, silver-impregnated¹⁰⁾, and heparin-coated catheters¹³⁾, as well as those providing sustained release of chlorhexidine¹¹⁾. Irrigation with antibiotics¹⁶⁾ or povidone-iodine solution²⁾ has also been suggested for the prevention of the urinary tract infections associated with urinary catheters. However, all these methods have failed to reduce the incidence of catheter-associated bacteriuria for patients catheterized for periods of longer than two weeks^{6,8,17)}. Therefore, the early removal of a urinary catheter is necessary to prevent urinary tract infections associated with urinary catheters.

The incidence of *E. coli*, *P. aeruginosa*, *S. marcescens* and *E. faecalis* of strains isolated from urinary tract infections at the Department of Urology, Hiroshima University Hospital is shown in Table 2. *P. aeruginosa* and *S. marcescens* were more frequent than *E. coli* and *E. faecalis* in the patients with nosocomial infections and indwelling urinary catheters at the Department of Urology, Hiroshima University Hospital. We therefore used *P. aeruginosa* and *S. marcescens* in this study.

Table 2. Incidence of *E. coli*, *P. aeruginosa*, *S. marcescens* and *E. faecalis* from urinary tract infections with nosocomial infections or indwelling urinary catheters at the Department of Urology, Hiroshima University Hospital from 1978 to 1984.

Bacterium	Isolated strains number		
	Total	Isolated from patients with	
		nosocomial infections	indwelling catheters
<i>E. coli</i>	273	26 (10)	7 (3)
<i>P. aeruginosa</i>	94	42 (45)	28 (30)
<i>S. marcescens</i>	81	67 (83)	59 (73)
<i>E. faecalis</i>	179	63 (35)	26 (15)

The numbers in parenthesis represent the percentage.

N-Acylated amino acids have been reported to remove *Candida albicans* from catheter materials more than other chelating agents and disinfectants¹⁴⁾. In the present study, we employed *P. aeruginosa* and *S. marcescens* to assess the abil-

ity of the *N*-acylated amino acids to remove adherent bacteria. Among the *N*-acylated amino acids tested in this study, glycine and phenylalanine with acyl residues of eight and ten carbons showed the greatest ability to remove adherent bacteria.

Although the mechanism by which *N*-acylated amino acids remove adherent bacteria is not clear, their surfactant and chelating characteristics might be involved. This is because *N*-acylated amino acids have almost no antibacterial activity (data not shown) and their chemical formula like low molecular soap. Octyl phenylalanine has been used to promote the absorbance of ampicillin suppositories from the rectum, which indicates that it is less toxic than disinfectants. In addition, no severe side effects of octyl phenylalanine have been reported as far as we know.

Our findings suggest that *N*-acylated amino acids are useful compounds in removing adherent bacteria to catheter materials and new type agents may possibly prevent catheter-associated urinary tract infection.

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