Surface Modification of Poly(styrene) by the Attachment of an Antimicrobial Peptide

P. APPENDINI, J. H. HOTCHKISS

Department of Food Services, Stocking Hall, Cornell University, Ithaca, New York 14853

Received 3 April 2000; accepted 17 July 2000

ABSTRACT: Polymers that directly inhibit the growth of microorganisms at their surface are potentially useful. To investigate the feasibility of such materials, poly(styrene) (PS) resin beads that had had poly(ethyleneglycol) (PEG) grafted onto the surface were further derivatized by covalently linking an antimicrobial peptide onto the surface. The antimicrobial peptide was composed of 8 lysine and 7 leucine (6K8L) residues. The resulting surface-modified polystyrene (SMPS) was microcidal in a concentration and time-dependent manner against several micro-organisms including *E. coli* O157 : H7, *L. monocytogenes, S. aureus, P. fluorescens,* and *K. marxianus* when suspended in phosphate buffer. The SMPS inhibited the growth of pathogenic *E. coli* O157 : H7 in trypticase soy broth. SMPS was bactericidal at pH 3.5 to 7, retained activity after heating to 200°C for 30 min, and could be extensively washed without loss of antimicrobial activity. Bioassays and HPLC analyses of buffer that had been preincubated with SMPS indicated that antimicrobial activity may have been due, at least in part, to the slow release of a peptide–PEG ligand from the PS to the buffer. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 609–616, 2001

Key words: antimicrobial polymer; cationic peptide; surface modified polystyrene; food-borne micro-organisms; antimicrobial packaging

INTRODUCTION

Several methods to render synthetic polymers antimicrobial have been investigated. The goal has been to use these polymers in applications such as biomedical devices including implants and sterile packaging, food and pharmaceutical processing equipment, aseptic environments, and food-contact packaging.[1–3] Examples include synthetic polymers containing quaternary ammonium or phosphonium salts,[4–6] quinolates,[7] and a variety of metals such as copper, vanadium,[8] and silver,[9] among others. Generally, polymers containing such additives are not permitted for direct food contact due to the potential migration of nonapproved substances to foods, and may not be suitable for some biomedical applications such as implants.

Fungicides and food-approved antimicrobials have been incorporated into polar and nonpolar polymers.[10–13] Most antimicrobial polymers are only effective when the antimicrobial agents are released from the polymers. Antimicrobial polymers that do not release compounds to the environment have also been investigated,[14–18] but it is unclear if microbial death occurred or if the micro-organisms were simply adsorbed onto the polymer surface. Compounds of "natural" origin such as nisin and lysozyme have also been incorporated into polymers.[19–23] UV-irradiation of ny-

Correspondence to: J. H. Hotchkiss.

Contract grant sponsors: Consejo nacional de Ciencia y Tecnologia and the Department of Food Science (Cornell University).

Journal of Applied Polymer Science, Vol. 81, 609–616 (2001)

lon 6,6 film was reported to impart antimicrobial activity due to the formation of surface-bound amines.[24]

A major limitation of polymers containing natural antimicrobials is the relatively narrow spectra of organisms against which these compounds are effective, as well as their low activity. Water-soluble naturally occurring and synthetic peptides have shown potent antimicrobial activity against a wide range of clinical[25] and food-borne[26] micro-organisms.

Our objective was to synthesize and assess the antimicrobial activity of poly(styrene) to which a synthetic antimicrobial peptide had been grafted on a range of micro-organisms, and to determine if the activity was due only to bound peptide or if peptide derivatives were released from the poly-(styrene). The effect of pH and heat on antimicrobial activity was also investigated.

METHODOLOGY

Materials

BBL trypticase soy broth (TSB), trypticase soy agar (TSA), and standard methods agar were obtained from Krackeler Scientific Inc. (Albany, NY). All other chemicals were of analytical or reagent grade.

Bacterial Strains

The following strains were used in the antimicrobial activity assays: *Bacillus subtilis* (wild-type PB2, 168 Marburg strain[27]), *Escherichia coli* O157 : H7 (ATCC 33150; Rockville, MD), *Kluyveromyces marxianus* (cheese isolate; Food Science Dept., Cornell University), *Listeria monocytogenes* (ATCC 689426; Rockville, MD), *Pseudomonas fluorescence* (milk isolate; Food Science Dept., Cornell University), *Salmonella typhimurium* H 3380 phage type DT 104, *Serratia liquefasciens* (skim milk isolate; Food Science Dept., Cornell University), and *Staphylococcus aureus* (ATCC 13566; Rockville, MD). Cells were maintained frozen in trypticase soy broth (TSB) containing 10% glycerol or refrigerated in trypticase soy agar (TSA) slants. A loop full of was transferred from TSA and grown overnight at 25°C in rotating flasks (100 rpm) containing 20 mL tripticase soy broth (TSB). A 50-μL aliquot from the overnight culture was transferred to fresh TSB and grown at 25°C to mid-exponential or in some cases stationary phase of growth. The cells were centrifuged, washed, and serially diluted in phosphate buffer (pH 7.2) to be used as inocula.

Modification of Polystyrene Surface

Surface-modified poly(styrene) (SMPS) was synthesized from 90 μm diameter low crosslinked poly(styrene) (with 1% DVB) beads (Calbiochem-Novabiochem Corp, San Diego, CA) onto which a derivatized polyethylene glycol (PEG) spacer was grafted at the surface. The PEG was attached to the PS by anionic polymerization of ethylene oxide using a potassium salt of poly(stylmethyltetraethylene glycol ether). The PS was nonporous and, hence, the PEG was grafted only at the surface. The PEG acted as a spacer between the PS and the peptide. A peptide with the sequence HOOC-leu-lys-leu-leu-lys-lys-leu-leu-lys-leu-leu-lys-lys-leu-NH_3[25] was synthesized by solid-phase peptide synthesis (SPPS) at the terminal end of the PEG spacer after the PEG had been attached to the PS. The synthesis was performed using Fmoc (9-fluorenylmethoxycarbonyl) chemistry.[28] The carboxyl end of the first amino acid residue (leucine) was esterified to the free end of the PEG. Coupling of each subsequent amino acid was accomplished using 2-(H-benzotriazole-1-yl)-1,1,3,3 tetramethyluronium tetrafluoroborate (TBTU), 1-hydroxybenzotriazole (HOBt) and N-methylmorpholine (NMM). The reaction protocol was as follows: Fmoc deprotection with 20% piperidine in *N,N*-dimethylformamide (DMF), washing with DMF, and coupling by using a threefold excess of Fmoc amino acid/TBTU/HOBT in the presence of NMM. After the synthesis, the PS with grafted PEG–peptide was washed with methanol and ether and dried *in vacuo*. The dry protected peptide–PS resin was treated with TFA at room temperature to remove the protecting groups. The PS resin with attached peptide (surface-modified polystyrene; SMPS) was filtered and washed with each, TFA, methanol, and water.

The amount of peptide attached to the PS was determined by HPLC after hydrolysis of the peptide from the PS, using a Waters PicoTag (Mildford, MA) amino acid analysis system. The peptide was hydrolyzed from the resin with 6 *N* HCl at 150°C for 95 min. The amino acids were derivatized by the phenylisothiocyanate (PITC) method and analyzed by RP-HPLC.[29,30] The resulting phenylthiocarbamyl amino acid derivatives were separated on a 4.6 $\times$ 300 mm Nova Pack C_{18} column employing a modified Pico-Tag

buffer system. The RP-HPLC mobile phases were 0.1% TFA (Applied Biosystems, HPLC grade) in water (Milli-Q; millipore, Bedford, MA) and 0.1% TFA in acetonitrile.

Antimicrobial Activity

Surface-modified poly(styrene) (SMPS) was suspended in 1 mL of phosphate buffer (pH 7.2) at concentrations of 1, 5, 10, 20, 40, and 60 mg/mL. SMPS suspensions were inoculated with mid-exponential cells to give counts of 10^6–10^7 CFU/mL and incubated in 1 mL microtubes with snap lids at 25°C in a rotator (Mallinkrodt, St. Louis, MO). Samples were taken at 10, 30, and 60 min, diluted in phosphate buffer (pH 7.2), and enumerated on TSA by standard pour plate methods.[31] Cells added to phosphate buffer alone and phosphate buffer containing PS-PEG resin without attached peptide were treated the same and served as controls.

To test the effect of the SMPS in growth medium, 10^4, 10^6–10^7 CFU/ml mid-exponential *E. coli* O157 : H7 were incubated with SMPS (5, 10, 20, and 40 mg/mL) in TSB at 25°C in a rotator (Mallinkrodt, St. Louis, MO). Samples taken at 0, 2, 4, 8, and 18 h and enumerated on TSA by standard pour plate.[31] TSB cell suspensions without PS and with PS-PEG to which peptide had not been grafted served as controls.

Effect of pH on Antimicrobial Activity

Mid-exponential *E. coli* O157 : H7 were inoculated into 0.1*M* citrate buffers of pH 3.5–7 to final concentrations of 10^6–10^7 CFU/mL. The suspensions were incubated with 20 mg/mL of SMPS at 25°C. After 10 min, 0.1-mL aliquots were enumerated in triplicate by pour plate on TSA.[31] One-way analysis of variance and the Tukey test determined significant differences between the cell count means.

Effect of Temperature on Antimicrobial Activity

SMPS was subjected to the following heat treatments: 121°C, 20 min (autoclave); 120°C, 60 min (dry oven); 200°C, 30 min (dry oven); 400°C, 15 min (dry oven). Heat-treated SMPS was then suspended in phosphate buffer or TSB at 40 mg/mL, inoculated with *E. coli* O157 : H7 and incubated at 25°C. Aliquots of 0.1 mL were taken over time, diluted, and enumerated by enumerated by standard methods on TSA.[31]

Release of Peptide from SMPS

SMPS was suspended in phosphate buffer (40 mg/mL) for 24 h at 25°C and separated from the buffer by centrifugation. Aliquots of the resulting buffer were diluted 1 : 5, 1 : 10, 1 : 20, and 1 : 40 (v/v) with fresh buffer and the dilutions inoculated with 10^7 CFU/mL of *E. coli* O157 : H7. After 30 min, cell numbers were enumerated by dilution and pour plate.[31] Additional aliquots of buffers that had been incubated with SMPS were centrifuged through molecular filters (Microcon; Millipore Corp, MA) of 3000, 10,000, and 30,000 Da molecular weight cutoffs. The filtrates were incubated with *E. coli* O157 : H7 (10^7 CFU/mL) at 25°C for 30 min, then diluted and enumerated by standard methods.[31]

Forty milligrams of SMPS was suspended in 1 mL of buffer, agitated for 24 h, and centrifuged to separate the SMPS from the buffer. This process was repeated 40 times with the same 40 mg of SMPS. The first 15 samples of buffer were pooled and tested for antimicrobial activity against *E. coli* O157 : H7 as described above. After 25 additional washes (total = 40), the solid SMPS was also tested for antimicrobial activity against *E. coli* O157 : H7, as described above.

The combined supernatants from the repeated suspensions of SMPS described above were concentrated fivefold in a Speed Vac Concentrator (Savant, Holbrook, NY) and analyzed by RP-HPLC on a PE Biosystems 130 instrument (Applied Biosystems, Foster City, CA) using a Microbore C18, 2 × 100 mm column (YMC Inc., Willmington, NC). The compounds were eluted with a linear gradient of 5 to 60% water–0.1% TFA/acetonitrile–0.1% TFA over 40 min at a flow rate of 0.22 mL/min at 31°C. UV absorbance was monitored at 210 nm. Fractions from the HPLC were collected and tested for antimicrobial activity against *E. coli* O157 : H7 as described above. The fraction with highest antimicrobial activity was analyzed for amino acid content as described above.

RESULTS

Modification of Poly(styrene) Surface

Amino acid analysis of the peptide hydrolyzed from the PS resin indicated that 0.204 mmol of peptide was attached per gram of PS. Considering the molecular weight of the peptide given by the mass spectral analysis (1692.1 Da), it was esti-

mated that 345 mg of peptide were attached per g of dry PS resin. The PS resin is nonporous, and therefore, the peptide is attached only at the surface. One gram of resin comprises approximately 2.86×10^6 beads with a nominal diameter of 90 μm,[32] thus the amount of peptide per surface area was estimated to be 0.47 mg $\cdot$ cm^2.

Antimicrobial Activity

Three to 60 mg of SMPS per mL of buffer reduced the microbial counts of all organisms evaluated by 1 to 6 $\log_{10}$ CFU/mL after a 10, 30, or 60 min exposure (Fig. 1). *B. subtilis* was the most sensitive organism (5 mg SMPS/mL reduced counts to < 1 $\log_{10}$ CFU/mL after 10 min of exposure) followed by *L. monocytogenes, E. coli* O157 : H7, *P. fluorescence, S. liquefasciens,* and *S. typhimurium. S. aureus* was the most resistant organism. Although *S. aureus* was reduced by 1–2 $\log_{10}$ CFU/mL at 10 mg/mL and 30-min exposure, higher SMPS concentrations and longer exposures reduced counts by only 3–4 $\log_{10}$ CFU/mL. As little as 5 mg/mL of SMPS reduced *K. marxianus* (yeast) counts by 1–2 $\log_{10}$ CFU/mL after 10 min of exposure. *B. subtilis, L. monocytogenes,* and *E. coli* O157 : H7 were reduced by 6–7 $\log_{10}$ CFU/mL at SMPS concentrations of 5, 10, and 40 mg/mL buffer, respectively, after 60 min exposure. The numbers of viable cells in buffer did not change in the absence of SMPS or when 20, 40, and 60 mg/mL of control PS (without attached peptide) were added to the buffer.

Increasing the time of exposure to SMPS reduced the amount of SMPS required giving a similar reduction in cell counts (Fig. 1). For example, *K. marxianus, S. typhimurium,* and *E. coli* O157 : H7 were reduced 3–4 $\log_{10}$ CFU/mL by 10 mg/mL of SMPS after 60 min. A SMPS concentration of approximately 20 mg/mL was required to reduce counts by a similar amount in 10 min. SMPS (5 mg/mL) reduced *L. monocytogenes* counts to below minimum detection at 60 min, while 20 mg/mL was required for a similar cell count reduction in 10 min.

SMPS was also bactericidal against cells growing in TSB. For example, $> 10^4$ CFU/mL *E. coli* O157 : H7 were reduced to < 10 CFU/mL (the lower limit of enumeration) after 1 h at a SMPS concentration of 40 mg/mL (Fig. 2). The bactericidal activity of the SMPS was less pronounced in nutrient broth compared to buffer. SMPS concentrations of 10 mg/mL were initially bactericidal against *E. coli* O157 : H7, but growth resumed

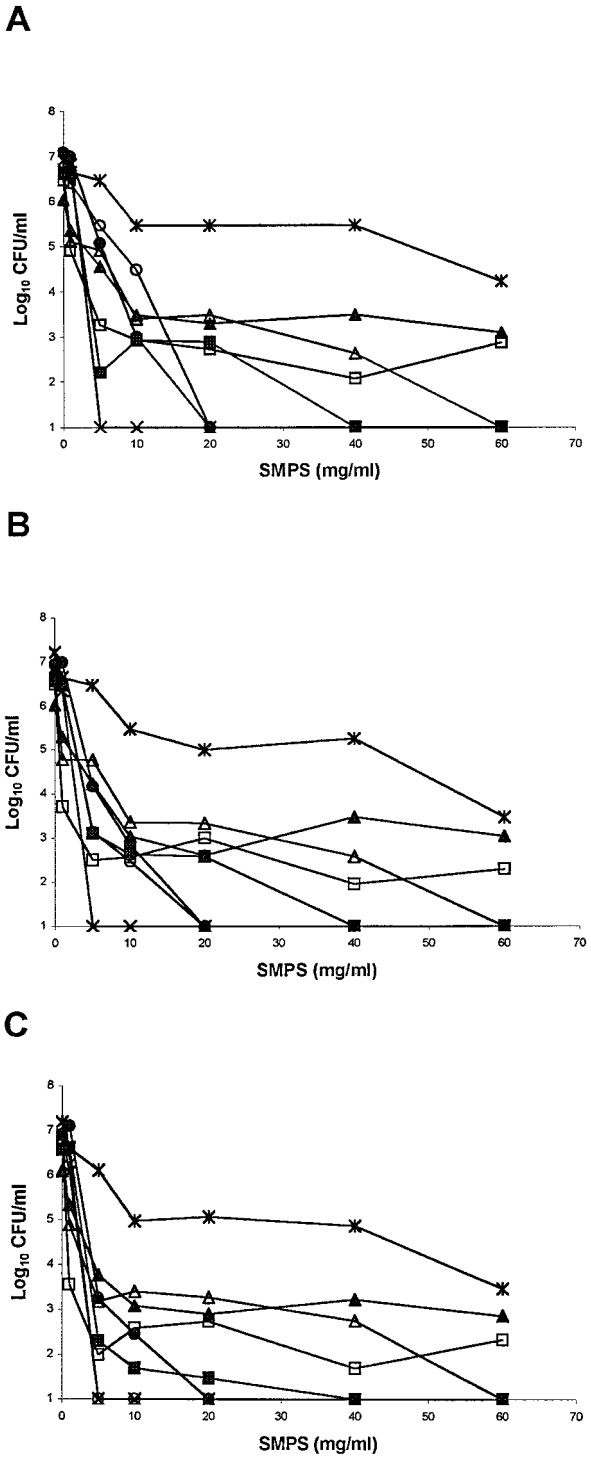

Figure 1 Effect of SMPS concentration on the viability of *B. subtilis* (×), *E. coli* O157 : H7 (■), *K. marxianus* (▲), *L. monocytogenes* (○), *P. fluorescence* (●), *S. typhymurium* (△), *S. liquefasciens* (□), and *S. aureus* (✳) suspended in phosphate buffer (pH 7.2) for 10 (A). 30 (B) and 60 (C) min at 25°C. The minimum dilution plated was 1×10^{-1}.

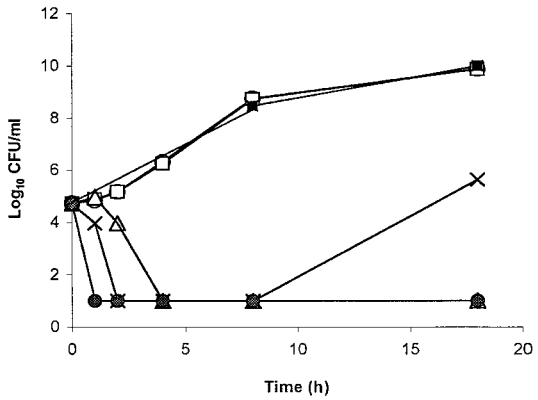

Figure 2 Effect of SMPS concentration on *E. coli* O157 : H7 growth in TSB at 25°C. The concentrations of SMPS in TSB were 0 mg/mL (○), 6 mg/mL (■), 10 mg/mL (×), 20 mg/mL (△), and 40 mg/mL (●). The concentration of resin without peptide was 40 mg/mL (□). The minimum dilution plated was 1×10^{-1}.

after 8 h and reached 10^6 CFU/mL at 18 h (Fig. 2). Concentrations of 6 mg/mL SMPS had no effect on numbers of *E. coli* O157 : H7 enumerated from growth medium, probably because at this SMPS concentration cell growth was greater than cell death. The cells also grew equally well in broth alone compared to broth containing unmodified PS.

Effect of Heat on SMPS Activity

Autoclaving the SMPS at 121°C for 15 min or dry heating at 120 and 200°C for 1 and 0.5 h, respectively, had no effect on the antimicrobial activity SMPS (Table I). Five $\log_{10}$ CFU/mL reductions in counts were observed for the *E. coli* O157 : H7 in TSB after the SMPS had been heated at 200°C for 30 min. At 400°C, the SMPS charred.

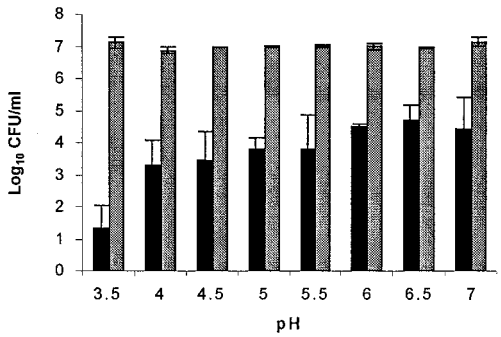

Figure 3 Antimicrobial activity of SMPS against *E. coli* O157 : H7 suspended in 0.1*M* citrate buffers pH 3.5–7.0. SMPS concentrations were 0 mg/mL (■ light shading) and 40 mg/mL (■). The minimum dilution plated was 1×10^{-1} CFU/mL.

Effect of pH on SMPS Activity

The SMPS remained antimicrobial against *E. coli* O157 : H7 over a pH range of 3.5–7 in citrate buffer (Fig. 3). Cell reduction was significantly ($p < 0.05$) greater at pH 3.5 than pH 7.

Release of Peptide from SMPS

A reduction in *E. coli* O157 : H7 counts of approx. 5 $\log_{10}$ CFU/mL counts was observed in sterile phosphate buffer that had been preincubated with SMPS for 24 h, the SMPS removed, and the remaining buffer inoculated with viable organisms. *E. coli* O157 : H7 counts were reduced one $\log_{10}$ when suspended in 1 : 5 and 1 : 10 (v/v) dilutions of these pretreated buffers. An initial *E. coli* O157 : H7 population of 10^7 CFU/mL was reduced by 1–3 $\log_{10}$ CFU/mL when suspended in buffers that had been incubated with SMPS and

Table I Effect of Heat Exposure of SMPS on Antimicrobial Activity in Growth Medium (TBS) and Buffer (CFU/mL)

	TSB[a]			Buffer[b]	
Incubation Time (h)	Control (No SMPS)	121°C Autoclave, 15 min	120°C Dry, 1 h	200°C Dry, 30 min	400°C Dry, 30 min
					Resin charred
0	1.06×10^4	1.06×10^4	1.3×10^5	4.7×10^6	
0.50				$< 10^1$	
4	2.52×10^6	$< 1 \times 10^2$	$< 1 \times 10^2$		
8	2.79×10^8	$< 1 \times 10^2$	$< 1 \times 10^2$		
12	1.06×10^{10}	$< 1 \times 10^2$	$< 1 \times 10^2$		

[a] Minimum dilution plated: 1×10^{-2} CFU/mL.
[b] Minimum dilution plated: 1×10^{-1} CFU/mL.

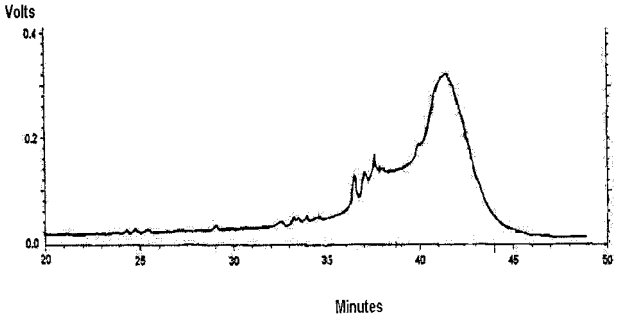

Figure 4 RP-HPLC analysis of 5× concentrated phosphate buffer previously incubated with SMPS. Numbers indicate fractions corresponding to those in Table IV. Injection time was 10.6 min.

filtered through 10,000 and 30,000 Da molecular weight cutoff membranes. No significant reduction in *E. coli* O157 : H7 counts were observed when the cells were suspended in buffers that had been filtered through 3,000 Da MWC membranes.

RP-HPLC analyses of the concentrated buffer that had been previously incubated with SMPS for 24 h showed several peaks with retention times (Rt) of 18 to 40 min (Fig. 4). RP-HPLC fractions were collected from Rt of 18.5 to 30.9 min and assayed for antimicrobial activity. The highest activity was obtained with the 26.5, 27, and 31 min RT fractions (Table II), while the fraction corresponding to authentic soluble peptide (RT 26 min; fraction 5) showed no antimicrobial activity (Table II). Amino acid analysis of the 27 min RT sample (fraction 7) showed leucine and lysine present at a molar ratio of 4 : 3.

DISCUSSION

(Poly)styrene was rendered antimicrobial by grafting a cationic, amphipathic peptide to its nonporous surface using PEG as a spacer molecule. Poly(styrene) was chosen based on its potential high loading capacity (0.24 mmol/g) and ease of surface activation.

The surface modified polystyrene (SMPS) was antimicrobial against all organisms tested, including yeast, Gram positive, and Gram negative bacteria. *S. aureus* was the least susceptible to the SMPS. The reason for the relatively low susceptibility of *S. aureus* is unknown, but was apparently not related to inherent characteristics of the peptide or the micro-organism because *S. aureus* has been shown to be susceptible to soluble peptide concentrations as low as 10 μg/mL.[24] SMPS was also antimicrobial against pathogenic *E. coli* O157 : H7 in TSB. Activity was directly related to the concentration of SMPS and time of exposure. Unmodified PS did not cause reduction or inhibition in any organism.

SMPS remained bactericidal after being washed 40 times in phosphate buffer. The buffer used to wash the SMPS was also bactericidal, suggesting that the peptide and/or other antimicrobial components detached from the surface of the PS. Free peptide concentrations as low as 10 μg/mL are bactericidal.[24] Twenty milligrams of SMPS contains approximately 6,900 μg of peptide. If 0.2% of the peptide from 20 mg of SMPS were hydrolyzed from the PS, the concentration of soluble peptide would be 14 μg/mL in a 1-mL culture. This is above the concentration required to be antimicrobial, indicating that only small amounts of the peptide need be released to show antimicrobial activity.

It has been suggested that synthetic peptides grafted onto polyamide were antimicrobial without being released into the growth media.[25] However, only experiments using growth media were employed, and would not have detected the activ-

Table II Antimicrobial Activity of Peptide Standards and Fractions Collected from HPLC Separations of Compounds Desorbed from the SMPS

Sample	Retention Time (min)	Antimicrobial Activity[a]
Peptide standard[b] (μg/mL)		
100	26.03	++
10	26.04	++
5	25.87	++
Fraction number		
1	18.54	−
2	22.68	−
3	22.94	−
4	23.40	−
5	25.95	−
6	26.50	+
7	27.03	++
8	30.90	+

[a] Activity against 4.5×10^6 CFU/mL. *E. coli* O157 : H7 in buffer. ++ Reductions higher than 4 $\log_{10}$; + reductions lower than 4 $\log_{10}$; − no reduction.

[b] Standards were 1000, 100, 10, and 5 μg/mL of soluble peptide in buffer.

ity of small amounts of peptide released from the resin. When conducted in growth media, antimicrobial activity can be masked by rapid growth. While experiments in media represent the sum of growth and death, tests in buffer represent only cell death. Experiments in buffer that had been preincubated with SMPS and the SMPS removed, confirmed that antimicrobial activity was being released from the SMPS. The 1 : 5 and 1 : 10 dilutions of the buffers that had been incubated with SMPS prior to inoculation were bactericidal against *E. coli* O157 : H7 after the SMPS had been removed. Higher dilutions (1 : 20 and 1 : 40) of the buffer were not antimicrobial. Buffers that were preincubated with SMPS and filtered through 10,000 and 30,000 Da molecular weight cutoff membranes prior to inoculation were antimicrobial, whereas those filtered through 3000 Da were not. This indicated that antimicrobial compound(s) with MW $>$ 3,000 and $<$ 10,000 Da were detached from the SMPS. Such compounds would be of higher MW than the peptide, which has a MW of 1691 Da. The release of components from the SMPS into the buffers was confirmed by RP-HPLC analyses, which yielded a chromatogram with several peaks not seen in the free peptide chromatogram (Fig. 4). Fractions collected from the RP-HPLC were tested for antimicrobial activity. Peaks with retention times (26.5, 27, and 31 min) greater than the peptide (26 min) were antimicrobial.

The RP-HPLC and MWC membrane results showed that antimicrobial compounds with MW greater than the peptide alone were released from the SMPS. It is likely that the antimicrobial activity was due to hydrolysis of the PEG spacer with the peptide attached. The PS resin was functionalized with a PEG chain of 3000–4000 Da.[32] It has been reported that PEG can develop peroxides and chain cleavage.[33] Detachment of PEG chains with peptide attached from the PS would explain the presence of leucine and lysine at proportions equivalent to those of the free peptide in the fractions with a retention times greater than free peptide.

The activity of SMPS against *E. coli* O157 : H7 at pH 3.5 to 7.0 combined with the temperature stability suggests potential applications a wide range of applications. Because the SMPS maintained its activity after being subjected to 200°C, it would likely resist temperatures used in polymer processing such as stretch blow molding and thermoforming.

The authors would like to thank the Biotechnology Bioresource Center, Cornell University for their assistance with the peptide synthesis and amino acid analyses. Paola Appendini was supported by Consejo nacional de Ciencia y Tecnologia (CONACYT) in Mexico, and by the Department of Food Science at Cornell University.

REFERENCES

1. Price, D. L.; Sawant, A. D.; Ahearn, D. G. J. Indust Microbiol 1991, 8, 83. Intili, H. S. U.S. Patent 4533 435 (1985).
2. Rice, J. Food Process 1995, 56, 57.
3. Wood, P.; Jones, M.; Bhakoo, M.; Gilbert, P. Appl Environ Microbiol 1996, 62, 2598.
4. Kenawy, E. R.; Abdel–Hay, F. I.; El-Raheem, A.; El-Shanshoury, R.; El-Newehy, M. H. J Controlled Release 1998, 50, 145.
5. Chen, L.; Ma, W. Y.; Yu, X. H.; Yang, C. Z. Chin J Polym Sci 1996, 14, 205.
6. Bankova, M.; Manolova, N.; Markova, N.; Radoucheva, T.; Dilova, K.; Rashkov, I. J Bioact Compat Polym 1997, 12, 294.
7. Pancholi, H. B.; Patel, N. M. J Polym Mater 1996, 13, 261.
8. Nomiya, K.; Tsuda, K.; Sudoh, T.; Oda, M. J Inorg Biochem 1997, 68, 39.
9. Vojdani, F.; Torres, A. J Food Proc Preserv 1989, 13, 417.
10. Shetty, K. K.; Dwelle, R. B. Am Potato J 1990, 67, 705.
11. Weng, Y. M.; Hotchkiss, J. H. Pack Technol Sci 1993, 6, 123.
12. Ishitani, T. In Foods and Packaging Materials—Chemical Interactions; Ackermann, P.; Jaegerstaad, M.; Ohlsson, T., Eds., Royal Society of Chemistry: Letchworth, UK, 1995, p. 177.
13. Han, J. H.; Floros, J. D. J Plastic Film Sheeting 1997, 13, 287.
14. Pardini, S. P. U.S. Pat. 4708 870 (1987).
15. Olstein, A. D. U.S. Pat. 5142 010 (1992).
16. Cuq, B.; Gontard, N.; Guilbert, S. In Active Food Packaging; Rooney, M. L., Ed.; Blackie Academic & Professional: Glascow, 1995, p. 111.
17. Paik, J. S.; Kelley, M. J. IFT Annu Meet Abstr 1995, 93.
18. Hagelstein, A. E.; Hoover, D. G.; Paik, J. S.; Kelley, M. J. IFT Annu Meet Abstr 1995, 15.
19. Daeschel, M. A.; McGuire, J. U.S. Pat. 5451 369 (1995).
20. Appendini, P.; Hotchkiss, J. H. Pack Technol Sci 1997, 10, 271.
21. Ming, X.; Weber, G. H.; Ayers, J. W.; Sandine, W. E. J Food Sci 1997, 62, 413.
22. Cooksey, K.; Wu C. C. (Abstracts of Papers) Am Chem Soc 1998, 215.

23. Siragusa, G. R.; Cutter, C. N.; Willett, J. L. Food Microbiol 1999, 16, 229.
24. Shearer, A. E. H.; Paik, J. S.; Hoover, D. G.; Haynie, S. L.; Kelley M. J. Biotechnol Bioengin 67, 141.
25. Haynie, S. L.; Crum, G. A.; Doele, B. A. Antimicrob Agents Chemother 1995, 39, 301.
26. Appendini P.; Hotchkiss, J. H. J Applied Micro, 87, 750.
27. Piggot, P. G. J Bacteriol 1973, 114, 1241.
28. Fields, G. B.; Noble, R. L. Int J Protein Res 1994, 35, 161.
29. Heinrickson, R. L.; Meredith, S. C. Anal Biochem 1984, 136, 65.
30. Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. J Chromatogr 1984, 336, 93.
31. Speck, M. L. Compendium of Methods for the Microbiological Examination of Foods; American Public Health Association, Inc.: Washington, DC, 1984, p. 66.
32. Novabiochem 1999. Catalog and peptide Synthesis Handbook; Calbiochem-Novabiochem Corp: San Diego, CA.
33. Zalipsky, S.; Harris, J. M. Poly(ethylene glycol): Chemistry and Biological Applications; American Chemical Society Symposium Series 680. American Chemical Soceity: Washington, D. C., 1997.