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Development of a Peptide-containing Chewing Gum as a Sustained Release Antiplaque Antimicrobial Delivery System

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ABSTRACT

The objective of this study was to characterize the stability of KSL-W, an antimicrobial decapeptide shown to inhibit the growth of oral bacterial strains associated with caries development and plaque formation, and its potential as an antiplaque agent in a chewing gum formulation. KSL-W formulations with or without the commercial antibacterial agent cetylpyridinium chloride (CPC) were prepared. The release of KSL-W from the gums was assessed in vitro using a chewing gum apparatus and in vivo by a chew-out method. A reverse-phase high-performance liquid chromatography method was developed for assaying KSL-W. Raw material stability and temperature and pH effects on the stability of KSL-W solutions and interactions of KSL-W with tooth-like material, hydroxyapatite discs, were investigated.

KSL-W was most stable in acidic aqueous solutions and underwent rapid hydrolysis in base. It was stable to enzymatic degradation in human saliva for 1 hour but was degraded by pancreatic serine proteases. KSL-W readily adsorbed to hydroxyapatite, suggesting that it will also adsorb to the teeth when delivered to the oral cavity. The inclusion of CPC caused a large increase in the rate and extent of KSL-W released from the gums. The gum formulations displayed promising in vitro/in vivo release profiles, wherein as much as 90% of the KSL-W was released in a sustained manner within 30 minutes in vivo. These results suggest that KSL-W possesses the stability, adsorption, and release characteristics necessary for local delivery to the oral cavity in a chewing gum formulation, thereby serving as a novel antiplaque agent.

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INTRODUCTION

Dental plaque (oral biofilm) formation is a dynamic and complex process involving many stages, from the adsorption of salivary pellicle to bacterial accumulation and growth. More than 300 bacterial species that inhabit the oral cavity participate in this process.[1,2] Dental caries and periodontal diseases are closely associated with the development of dental plaque, formed as a result of the complex interactions between teeth and adsorbed host and bacterial molecules, passive transport of oral bacteria, co-adhesion of successive bacterial strains, and the multiplication of associated microorganisms.[3,4] Dental caries are initiated by bacterial adhesion and subsequent plaque formation on the tooth surface, followed by bacterial carbohydrate fermentation and organic acid formation. These acids diffuse into and ultimately demineralize the tooth.[5] For periodontal diseases, the presence of pathogenic plaque, together with host-related factors that modify the response to plaque bacteria, is the key determinant contributing to the development of the diseases.[6]

The pellicle formed as a result of selective adsorption of salivary proteins can influence early bacterial attachment and affect the acquisition of diseased or healthy microflora on the tooth.[7] In order to colonize, bacteria must first adhere to the tooth surface and then resist the cleansing forces of flowing saliva, lips, and cheeks.[7,8] Following the attachment of the pioneer colonizers, subsequent layers may form by cell-to-cell adherence and by the proliferation of adhering bacteria. Several bacterial and host factors are involved in the establishment of a balanced or climax microbial community.[9]

Two of the most common diseases of humans, caries and inflammatory periodontal disease, are exacerbated by the accumulation of bacterial plaques on tooth surfaces. The dental profession has developed preventive and therapeutic

regimens for these diseases based on the mechanical removal of the plaque. With the aim of preventing the incidence of caries, several investigators have reported attempts at inhibiting plaque accumulation on the surfaces of teeth and dental restorations by antibacterial varnishes or resinous materials containing antibacterial agents. In most of these formulations, water-soluble bactericides such as chlorhexidine were employed, and their effect was attributed to the release of the agent from the formulation.[10] However, antibacterial varnishes and resins that release bactericides have an intrinsic disadvantage in that the effect is transient and greatly diminishes over time. Nevertheless, there remains significant interest in the use of chemicals to replace or augment therapeutic procedures in dentistry. The development of antimicrobial agents for controlling growth of cariogenic and periodontopathogenic bacteria continues to be an area of interest.[11]

With a focus on the development of novel antimicrobial agents for the prevention and treatment of plaque-related oral diseases, naturally occurring antimicrobial peptides have emerged as one alternative class of antimicrobials. These antimicrobial peptides generally exhibit selective toxicity for prokaryotes and are not often associated with inducing microbial resistance. In order to develop more effective, broad-spectrum peptide antimicrobials, investigators have developed peptide libraries by using synthetic combinatorial technology. A novel decapeptide, KKVVFKVKFK (KSL), shows broad-spectrum antibacterial activity. The antiplaque effect of KSL has been tested and documented. Leung et al[12] reported that KSL may be a useful antimicrobial agent for inhibiting the growth of oral bacteria that are associated with caries development and early plaque formation.

In a previous report, KSL formulated in a chewing gum was effective as a novel antiplaque delivery system.[13] Unfortunately, KSL was degraded by peptide bond cleavage at Lys6-Val7 in human saliva, and at Phe5-Lys6 in simulated gastric fluids. Accordingly, 3 peptide analogs were prepared and evaluated in order to improve peptide stability, while maintaining antibacterial activity.[14] Of these, the peptide KSL-W was the most stable to salivary trypsin-catalyzed cleavage in the oral cavity, its proposed site of action, while it remained labile to hydrolytic degradation in simulated gastric fluid and by pancreatic enzymes. Thus, KSL-W, an L-tryptophan analog of KSL, wherein the L-Lys6 residue is replaced with L-tryptophan (H_2N-Lys-Lys-Val-Val-Phe-Try-Val-Lys-Phe-Lys-COOH), was selected as the most promising candidate in terms of potential therapeutic activity and safety in the gastrointestinal tract.[14]

Chewing gum may be a particularly effective means for delivering and maintaining a sufficient antibacterial dose of KSL-W in the oral cavity. Because chewing gum is usually kept in the mouth much longer than rinses and toothpastes, the active agent included in a chewing gum formulation, if efficiently released into the saliva, could exhibit sustained and improved delivery in the mouth.[15-17] As reported, the release of most water-soluble components from chewing gum is relatively rapid, often lasting no longer than 5 minutes. This release profile may not be sufficient for effective, sustained antiplaque treatment with water-soluble antimicrobials.[18,19] Therefore, improved formulations of chewing gum are required in order to provide sustained release of water-soluble components.

The objective of this study was to develop a novel, sustained release chewing gum formulation of the water-soluble antimicrobial peptide KSL-W for use as an antiplaque agent. Enzymatic and chemical stability of the peptide were evaluated at various pH levels and temperatures and the interaction of KSL-W with the primary mineral component of the teeth, hydroxyapatite, was examined. Chewing gum formulations containing KSL-W were prepared, and the release of KSL-W from the various gum formulations was studied in vitro using a commercial chewing apparatus and in vivo by a chew-out method. The overall goal of this research was to prepare an antimicrobial, antiplaque oral KSL-W delivery system for protecting against dental disease in those individuals unable to perform routine dental care, such as brushing, but who are otherwise able to chew gums. The gum was not intended to be a replacement for tooth brushing, but rather as an adjunct to routine dental hygiene.

Materials and Methods

Materials

KSL-W (lot 05US11311-B, molecular weight [MW] ~ 1307) was synthesized by SynPep (Dublin, CA). Peptide purity was confirmed by mass spectrometry with the molecular mass found at 1308.3. A commercial gum base was obtained from Fertin Pharma (Vejle, Denmark). Cetylpyridinium chloride (CPC), chlorhexidine, and benzalkonium chloride were purchased from Sigma, St Louis, MO. Hydroxyapatite (HA) spheroidal particles were obtained from BDH Chemicals Ltd, Poole, England. HA discs were purchased from Clarkson Chromatography Products Inc, South Williamsport, PA. Acetonitrile and methylene chloride (high-performance liquid chromatography [HPLC] grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Trifluoroacetic acid (TFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of analytical grade.

Peptide Determination

KSL-W was analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) using a Prosphere C-18 column (4.6 × 250 mm, Alltech, Deerfield, IL). A gradient elution using mobile phases consisting of 0.1% TFA in water

(A) and 0.1% TFA in acetonitrile (B) was performed as previously reported.[13] KSL-W was eluted after 12 minutes using a linear gradient running from 80:20 to 70:30 (A:B) at a flow rate of 1 mL/min; total run time was 16 minutes; injection volume was 40 μL. Chromatograms were recorded by UV detector at 215 nm. The correlation coefficient of the linearity for the detection of KSL was greater than 0.999 in a peptide concentration range of 20 to 400 μg/mL, and the assay was reproducible at these concentrations with a coefficient variation less than 5% (n = 3, intra-assay and interassay).

Benzalkonium Chloride, Chlorhexidine, and Cetylpyridinium Chloride Determination

Benzalkonium chloride, chlorhexidine, and CPC concentrations were determined by UV spectrophotometry using a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan). Serially diluted samples of artificial saliva were analyzed to give curves of absorption versus concentration. The absorption wavelengths employed for benzalkonium chloride, chlorhexidine, and CPC were 261.5, 253, and 259 nm, respectively.

Stability Studies

KSL-W Powder Stability

The stability of KSL-W powder was assessed at 4°C, 25°C (room temperature, RT), 40°C, and 60°C, and at 40°C in 90% relative humidity, for 1 year. At regular intervals, samples of the stored material were analyzed by HPLC and compared with samples of a frozen control. KSL-W stability was quantified by comparing the amount of intact KSL-W in the incubated samples to that in the corresponding controls.

KSL-W Solution Stability

Test solutions of KSL-W were prepared in deionized water. The degradation of KSL-W as a function of pH was then investigated by incubating standard volumes of the peptide solution in sodium acetate (pH 4), sodium phosphate (pH 7.4), and sodium borate (pH 9) buffers (0.1 M buffer concentration), as well as in artificial saliva (pH 5.7). Samples (10 mL) of each test solution, containing 500 μg/mL of KSL-W, were sealed in glass vials and incubated at 25°C, 37°C, or 55°C. Samples were taken at predetermined time intervals and analyzed by RP-HPLC as previously described.[13] The stability of KSL-W was also assessed in human saliva. Human saliva was collected from 4 healthy volunteers using institutional-approved procedures and passed through a 0.45-μm filter. KSL-W was dissolved in deionized water and then added to the saliva to give a final peptide concentration of 0.2, 0.5, or 1 mg/mL. The KSL-W-saliva solution was incubated at 37°C for 30 minutes. The amount of KSL-W remaining in the saliva after incubation was analyzed by RP-HPLC. KSL-W dissolved in water without saliva and incubated at 37°C was used as the control.

Stability of KSL-W After Incubation With Pancreatic Serine Proteases

The stability of KSL-W was assessed in vitro with 3 pancreatic serine proteases, α-chymotrypsin, elastase, and trypsin. The enzyme assays were developed based upon manufacturer specifications and the literature.[20] α-Chymotrypsin assays were performed in 40 mM Tris-HCl buffer (pH 7.8) containing 20 mM $CaCl_2$ and either 15 or 30 U of enzyme activity per milliliter. Elastase assays were performed in 40 mM Tris-HCl buffer (pH 8.0) containing either 1.25 or 2.5 U of enzyme activity per milliliter. Trypsin assays were performed in 40 mM Tris-HCl buffer (pH 8.0) containing 20 mM $CaCl_2$ and either 4000 or 8000 U of enzyme activity per milliliter. Each sample, incubated at 37°C, contained KSL-W (0.5 mg/mL) and the indicated amount of enzyme, expressed in units of activity. At regular time intervals over a 3-hour incubation, an aliquot part of the incubation mixture was placed in a sealed glass vial, immersed in boiling water for 45 seconds to stop further enzymatic activity, and assayed by HPLC. Concurrent control assays indicated that the peptides were not degraded by the buffer systems or the boiling water immersion, nor adsorbed to heat-treated enzyme. The data are presented as the mean ± standard deviation (SD) of 3 independent experiments.

Interaction of KSL-W With Hydroxyapatite Discs

The affinity of KSL-W for tooth-like materials was assessed by incubating a solution comprising 5 mg/mL KSL-W dissolved in 2 mL of artificial saliva with HA discs (4 discs, each with dimensions 0.38-inch diameter × 0.06-0.08-inch thick) at 37°C. The amount of free KSL-W adsorbed to the disc was calculated from the concentration of KSL-W remaining in solution after equilibrium. In order to assess the adsorption of KSL-W to HA discs that mimicked natural teeth present in the oral cavity, the HA discs were presoaked in filtered human saliva for ~1.5 hours at 37°C (4 HA discs in 4 mL of human saliva), and adsorption experiments similar to those performed with the uncoated HA discs were conducted. The 4 coated discs were weighed again, rinsed with artificial saliva, and transferred to 2 mL of artificial saliva containing 5 mg/mL KSL-W and incubated for 30 minutes at 37°C. Samples were withdrawn at different time intervals and analyzed for the amount of KSL-W adsorbed on the discs. Equilibrium adsorption was achieved after 30 minutes of incubation. As a control, untreated HA discs were directly added to KSL-W solutions at 37°C. The sample vials were mounted on a rotary wheel with vertical rotation at a speed

of 18 cycles/min. Samples were removed at predetermined times and centrifuged, and the supernatants were analyzed by RP-HPLC.

Adsorption Comparison of KSL-W, Benzalkonium Chloride, Chlorhexidine, and Cetylpyridinium Hydrochloride Onto Uncoated HA Discs

The adsorption of KSL-W, benzalkonium chloride, chlorhexidine, and CPC onto uncoated HA discs was studied by incubating 4 mL of ~0.43 mg/mL of each compound with 4 uncoated HA discs for 30 minutes at 37°C. The percentage adsorbed onto the discs was calculated by mass balance with the amount of the compound remaining in the supernatant solutions.

Chewing Gum Preparation

The chewing gum formulations were prepared using a standard technique. The gum base was softened at 65°C in a porcelain crucible. In the gums containing CPC, the CPC was added to the softened gum base and mixed for 2 minutes before adding any powdered ingredients. The KSL-W and xylitol were intimately triturated together with a spatula in a glass vial and then uniformly incorporated into the softened gum base by manual stirring with a metal spatula. After mixing, the homogeneous chewing gum was cooled to room temperature, molded into a rod of uniform shape, cut into pieces of identical weight, and hardened at room temperature overnight.

In Vitro Chewing Gum Release Studies

The in vitro dissolution and release of KSL from chewing gums was performed with a commercial gum chewing apparatus (AB FIA, Lund, Sweden) as previously described.[13,21] The apparatus is composed of 2 modules, each consisting of a thermostated glass test cell in which 2 vertically oriented pistons holding an upper and a lower chewing surface are mounted. The cells were filled with 20 mL of artificial saliva and maintained at 37°C. The ingredients of the artificial saliva were as follows: sodium chloride, 0.844 g; potassium chloride, 1.200 g; calcium chloride dihydrate, 0.193 g; magnesium chloride hexahydrate, 0.111 g; potassium phosphate dibasic, 0.342 g; water to make to 1000 mL. The pH was adjusted with hydrochloric acid solution to pH 5.7.[13] The chewing gum was placed between 2 inert nylon nets and inserted between the pistons onto the lower chewing surface. The chewing procedure consisted of up and down strokes of the lower surface combined with a twisting movement of the upper surface, thereby masticating the chewing gum and concurrently agitating the test medium. The chewing frequency employed in this study was 50 ± 2 strokes per minute. At predetermined time intervals, 400 µL aliquots of the supernatant, artificial saliva, were removed and assayed for KSL-W content by RP-HPLC. The release medium was replaced with fresh artificial saliva after each sample was taken.

Chew out In Vivo Gum Release Studies

In vivo chew out studies were performed, wherein 2 volunteers masticated 1 piece of gum at 30 to 40 chews/min. A typical American chews a piece of gum for 20 minutes.[17] As such, the chew out periods used in this study were 5, 10, 20, or 30 minutes. After chewing the gum for the indicated time interval, the gums were collected and assayed for the amount of KSL-W that remained. The remaining KSL-W was exhaustively extracted by heating the gum to 50°C to 60°C for 5 minutes, then adding 5 mL of a 1:1 mixture of acetonitrile and dimethylsulfoxide (DMSO) to the molten gum. After vigorously shaking for 5 minutes, 10 mL of 0.1 M acetate buffer (pH 4) was added, and the mixture was vigorously shaken for a further 30 minutes at room temperature. The sample was centrifuged to remove solids, the collected supernatant was filtered with a 0.45-µm filter, and the KSL-W content was assayed by RP-HPLC.

Results and Discussion

HPLC Analysis of KSL-W

Figure 1 represents the HPLC chromatogram of KSL-W. The assay was reproducible under these concentrations with a coefficient of variation less than 5% (n = 3 intra-assay and interassay). The HPLC method could be used to resolve intact KSL-W from the degradation compounds produced in sodium borate buffer (pH 9) at 55°C for 3 days. No attempt was made to identify degradation products or to determine a degradation pathway, as these would likely involve the same peptide bond hydrolysis and oxidation that was noted with the KSL analogs.[13,14]

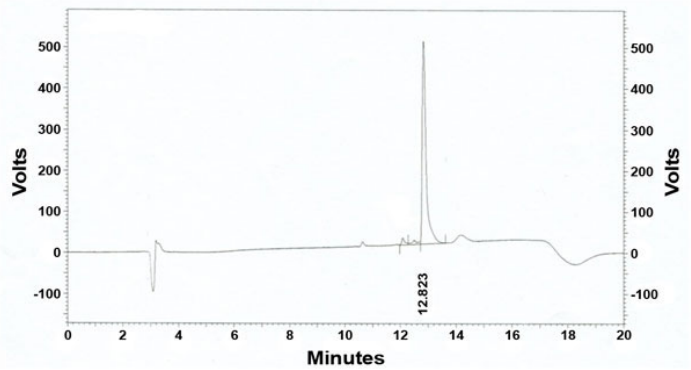

Figure 1. High-performance liquid chromatogram of KSL-W.

KSL-W Stability

Raw Material Stability

The stability of KSL-W powder stored under various conditions for 1 year is summarized in Figure 2. Under dry conditions, KSL-W was stable for 1 year at temperatures as high as 40°C. However, KSL-W was rapidly degraded under dry conditions at 60°C, and unstable at 40°C in 90% relative humidity. The half-life of KSL-W under these conditions, as estimated from Arrhenius plots, was 68.6 and 32.3 days, respectively. KSL-W appears to be particularly sensitive to moisture as seen in plots from Figure 2A and Figure 2B.

Chemical Stability in Aqueous Solutions

Figure 3 shows a plot of the amount of intact KSL-W remaining versus time after incubation in sodium acetate (pH 4), sodium phosphate (pH 7.4), and sodium borate (pH 9) buffers (0.1 M buffer concentration), as well as in artificial saliva (pH 5.7) and at 25°C and 37°C. The same experiments were performed at 55°C for which the data are not shown. However, the degradation constants given in Table 1 for 55°C demonstrate that KSL-W is less stable at this temperature. Degradation rate constants were obtained from the slope of the semilog plots of the concentration versus time data by

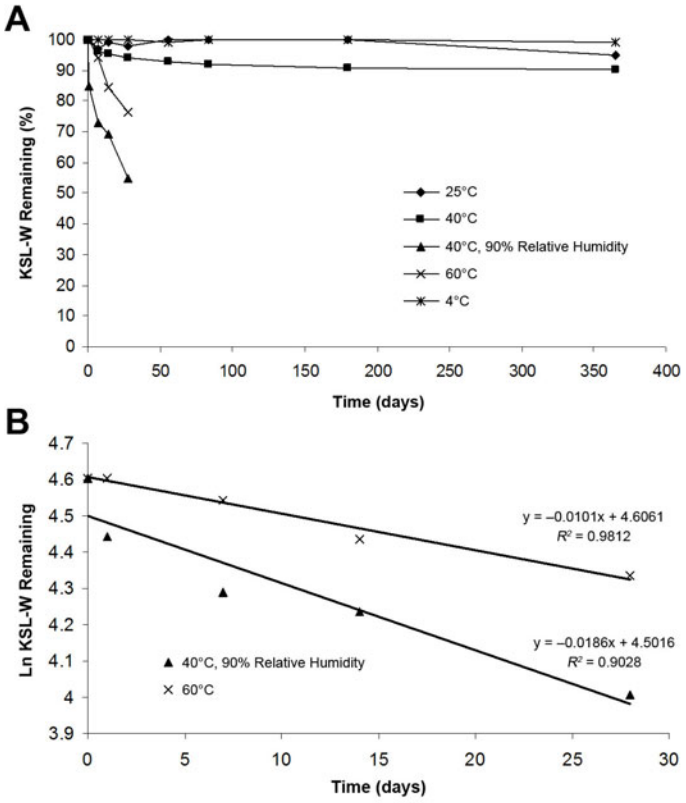

Figure 2. The stability of KSL-W stored under various conditions for 1 year (A) and a linear plot under accelerated conditions for 28 days (B).

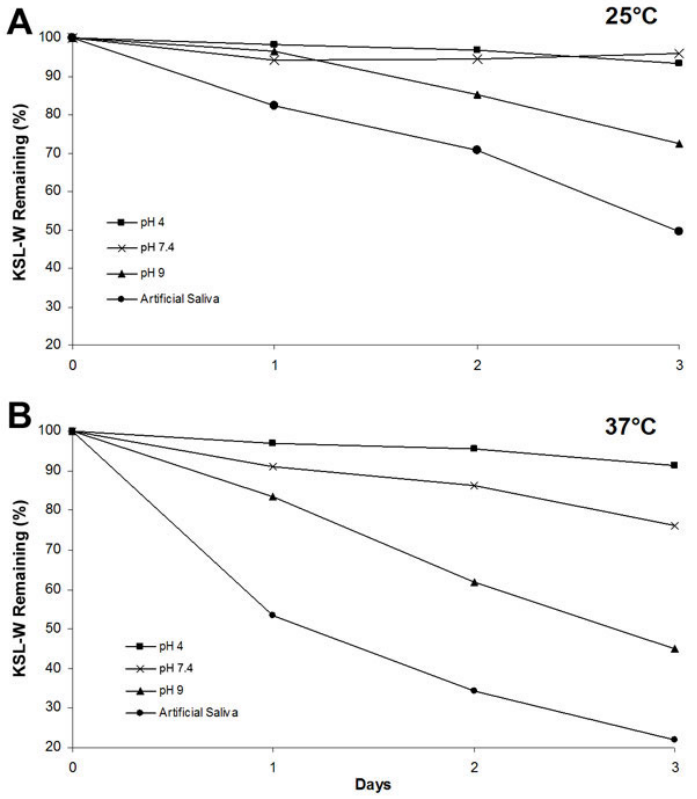

Figure 3. The effect of temperature and pH on the stability of KSL-W in solution at 25°C and 37°C.

regression analysis. The pH influenced the KSL-W degradation rate, and the observed degradation rates approximately followed first-order kinetics. The observed reaction rate constants of KSL-W are listed in Table 1. Although the optimum pH for KSL-W stability was not defined, the most favorable stability appeared to be in pH 4 acetate buffer. The half-life for KSL-W degradation at 37°C was 24.2 days at pH 4, 6.42 days at pH 7.4, 2.56 days at pH 9, and 9.57 days in artificial saliva (pH 5.7). Activation energies (Ea) derived from the slopes of these plots were 8.12 kJ/mol at pH 4, 26.07 kJ/mol at pH 7.4, and 27.03 kJ/mol at pH 9. After 24 hours, KSL-W degradation at 25°C and 37°C was 17.8% and 46.6%, respectively.

Stability to Pancreatic Serine Proteases

The percentage of KSL-W remaining after incubation at 37°C with a series of pancreatic serine proteases is shown in Figure 4. KSL-W, with an indole-containing L-tryptophan residue, was steadily degraded by α-chymotrypsin, which preferentially cleaves peptide bonds on the carboxylate side of an aromatic residue. After 3 hours, the percentage of KSL-W remaining in the 15 U/mL and 30 U/mL α-chymotrypsin digests was 63% and 17%, respectively. Elastase preferentially cleaves at the carboxylate side of peptide bonds containing small hydrophobic side chains and generally displays less selectivity than other serine proteases. KSL-W underwent

Table 1. Degradation Constants (day^{-1}) of KSL-W

Temperature (°C)	pH 4	pH 5.7 (Artificial Saliva)	pH 7.4	pH 9
25°C	22.4×10^{-3}	79.5×10^{-3}	53.1×10^{-3}	109.5×10^{-3}
37°C	28.6×10^{-3}	72.5×10^{-3}	108.0×10^{-3}	270.5×10^{-3}
55°C	30.6×10^{-3}	77.9×10^{-3}	143.2×10^{-3}	311.0×10^{-3}
Arrhenius equation	$y = -0.42 \times -0.21$	No dependence on temperature	$y = -1.36x + 3.34$	$y = -1.41x + 3.85$
Ea (kJ/mol)*	8.12		26.07	27.03

*Indicates activation energy.

modest elastase-catalyzed degradation. After 3 hours, the percentage of KSL-W remaining in the 1.25 U/mL and 2.5 U/mL digests was 85.9% and 70.1%, respectively. Trypsin hydrolyzes peptides at the carboxylate side of peptide bonds containing positively charged residues, such as the L-lysine residues contained in KSL-W. After 3 hours, modest KSL-W degradation was again observed, with 8% remaining in the 4000 U/mL digest and 76.1% remaining in the 8000 U/mL digest.

Interaction of KSL-W With HA

In a previous study,[14] the affinity of the KSL analogs to HA spheroidal particles was investigated by incubating an aqueous solution of the analogs with HA particles for 20 minutes at 37°C. The higher adsorption of KSL-W suggested that it should be more effective as an oral antiplaque agent than the KSL.

Interaction of KSL-W With HA Discs

The affinity (adsorption and desorption) of KSL-W for tooth-like materials and salivary proteins using uncoated or saliva-coated HA discs is shown in Figure 5. Equilibrium saturation adsorption occurred within 30 minutes. There was a discernible difference in KSL-W binding to the uncoated versus the saliva-coated HA discs. Approximately 13% of the KSL-W adsorbed to the uncoated HA discs, whereas more peptide was bound to coated surfaces. Since KSL-W is a cationic molecule containing 4 lysine residues, it may have significant potential for electrostatic interaction with the adsorbed acidic salivary glycoproteins present in saliva. This electrostatic interaction may account for the increased adsorption of KSL-W to saliva-coated HA discs.

The effect of saliva coating on KSL-W desorption was also substantial. Approximately 73% of the peptide adsorbed to the coated discs was released after 5 minutes. However, after equilibrium was reached in 30 minutes, ~40% of the initially adsorbed KSL-W remained, suggesting some readsorption. In contrast, 76% of the KSL-W adsorbed to the uncoated discs was released after 5 minutes, and only 23% of the total KSL-W adsorbed remained on the discs at equilibrium. Once again, this finding might be attributed to an interaction between the KSL-W and the macromolecules of human saliva that coated the discs. It has been strongly suggested that the retention of the cationic antiplaque agent chlorhexidine in the oral cavity is directly related to its capacity to inhibit plaque formation.[22-25] As shown in the present study, the affinity of KSL-W for saliva-coated or uncoated HA, coupled with its antimicrobial activity against plaque-forming oral bacteria, provides further evidence of its potential as an antiplaque agent.

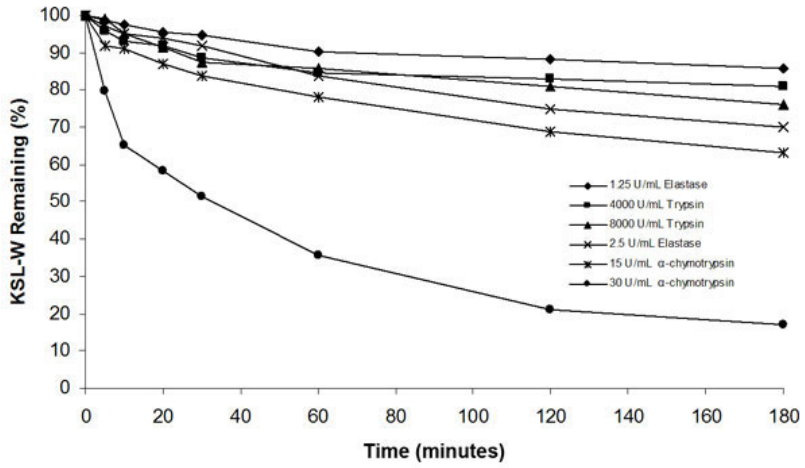

Figure 4. The stability of KSL-W incubated with pancreatic serine proteases at 37°C.

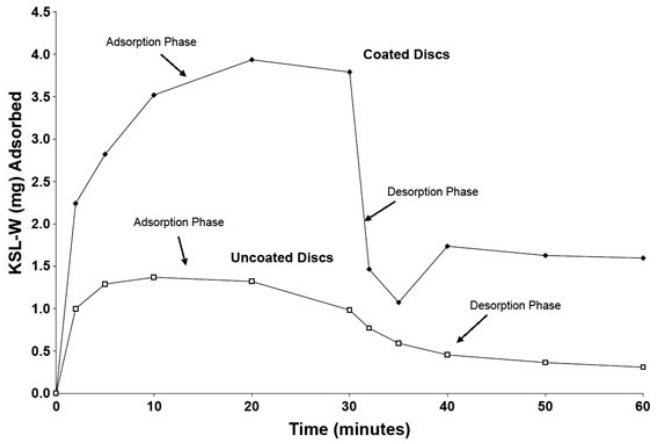

Figure 5. Adsorption-desorption interaction of KSL-W with hydroxyapatite (HA) discs.

Adsorption of KSL-W, Benzalkonium Chloride, Chlorhexidine, and CPC Onto Uncoated HA Discs

Chlorhexidine is considered to be one of the most effective plaque inhibitors. The efficacy of chlorhexidine against clinically significant microorganisms has been extensively explored.[26] It is reported that the surface chemical properties and HA adsorption characteristics of chlorhexidine are critical to its action as a plaque inhibitor. Accordingly, in order to further study the potential antiplaque characteristics of KSL-W, its adsorption to HA discs was compared with that of chlorhexidine and 2 other commercial antimicrobial agents, benzalkonium chloride and CPC.[27] Chlorhexidine adsorbed most avidly to the uncoated discs, some 33% more as compared with KSL-W. Nevertheless, KSL-W had a greater tendency to adsorb to HA than either benzalkonium chloride or CPC (Figure 6). It is clear that KSL-W exhibits significant adsorption to HA, an important characteristic for an antiplaque agent. These comparative adsorption data were applied in the design of formulation prototypes containing KSL-W and CPC.

In Vitro and In Vivo Release of KSL-W From Chewing Gum Formulations

Numerous devices and methods for studying in vitro drug dissolution and release from solid dosage forms have been developed.[28] However, these methods are not easily adapted to studying the release of drugs from chewing gums because continuous mastication is needed for modeling the drug release. The apparatus developed by Kvist et al is commonly employed for evaluating the in vitro release of drugs from medicated chewing gum formulations and was selected for evaluating KSL-W release from the formulations in the present study.[21] The gum formulations contained gum base 400 mg, xylitol 295 mg, and KSL-W 10 mg, with or without CPC 2 mg. Xylitol, a naturally occurring 5-carbon sugar alcohol, has gained considerable attention as an anticaries agent.[29] Noncariogenic xylitol is incorporated as a sweetener in chewing gums and tablets as well as in oral health care products such as dentifrices and mouth rinses. Although the mechanism of action is not fully clear, the beneficial effects of xylitol are generally explained by reduced acid formation and inhibition of xylitol-sensitive mutans streptococci.[30]

In vitro release studies of the chewing gum formulations were performed in artificial saliva in this study. It is known that artificial saliva does not have the same composition as human saliva. Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogeneous products, such as urea and ammonia. These components interact in related function in the following general areas: (1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva; (2) macromolecule proteins and mucins serve to cleanse, aggregate, and/or attach oral microorganisms and contribute to dental plaque metabolism; (3) calcium phosphate and proteins work together as an antisolubility factor; and (4) immunoglobulins, proteins, and enzymes provide antibacterial action.[31]

Figure 7 shows the in vitro and in vivo chew out release profiles of KSL-W from chewing gum formulations prepared with or without CPC. CPC is a quaternary ammonium compound with properties and uses typical of cationic surfactants. It exhibits antimicrobial activity against many oral bacteria. Because of its surface-active properties, it has a prolonged activity in the oral cavity as it is bound to the glycoproteins covering the teeth and oral mucosa. Studies evaluating 0.05% CPC formulations have shown variable results for plaque-inhibitory and antiplaque capacity.[32] In both cases, the inclusion of 2 mg CPC in the gum formulation caused an increase in the rate and amount of KSL-W released from the gum. After 90 minutes of chewing in vitro, ~90% of the KSL-W

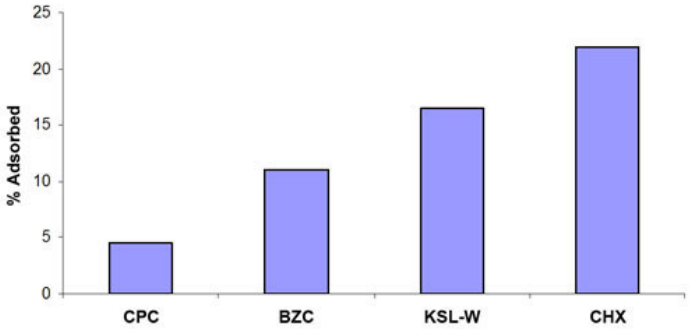

Figure 6. Comparative adsorption of cetylpyridinium chloride (CPC), KSL-W, chlorhexidine (CHX), and benzalkonium chloride (BZC) in artificial saliva onto uncoated hydroxyapatite (HA) discs at 37°C.

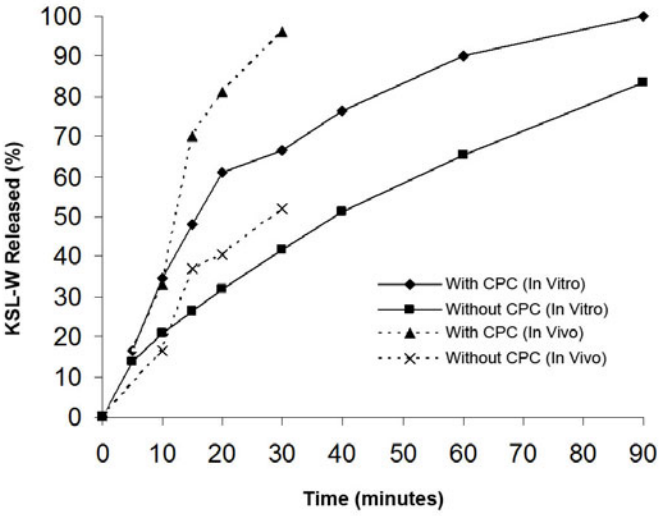

Figure 7. The effect of cetylpyridinium chloride (CPC) on the in vitro (solid line) and in vivo (broken line) release of KSL-W from gum formulations.

was released from the CPC-containing formulation. In contrast, less than 80% was released from the gum prepared without CPC. The same phenomenon was observed in vivo. At the end of a 30-minute chew out, cumulative KSL-W released from the CPC-free and CPC-containing gums was 52% and 96%, respectively. These results suggest that the inclusion of CPC in the gum may be particularly advantageous, as it increases the release rate of KSL-W and, as reported in the literature, also exerts significant surface active and antimicrobial activity in its own. Studies to assess the synergistic effects of CPC and KSL-W on their antimicrobial activity are ongoing. KSL-W remained stable throughout the formulation procedure and release studies, as only an intact KSL-W peak was detected during any HPLC analysis of the materials or release media (data not shown). In addition, the chewing gum formulations possess an acceptable taste owing to xylitol used as sweetener.

CONCLUSIONS

The antimicrobial peptide KSL-W has a strong affinity for human saliva-coated and uncoated HA and is sufficiently stable in human saliva to be used as an oral antiplaque agent. KSL-W is relatively stable across a wide pH and temperature range, which may simplify its formulation, storage, and use by consumers. KSL-W is likely to be degraded by gastric and pancreatic enzymes if used in vivo, thus limiting possible systemic absorption. KSL-W may be readily formulated into a variety of chewing gums, alone or in combination with established antiplaque agents, such as CPC. Both in vitro and in vivo studies revealed that KSL-W is released from chewing gums in a controlled and reproducible manner, suggesting that it is likely to be released and retained in the oral cavity. The inclusion of CPC in a KSL-W gum formulation further improves the rate and extent of KSL-W release.

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