JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 9005–9009 **9005**

Curcuma longa L. Constituents Inhibit Sortase A and *Staphylococcus aureus* Cell Adhesion to Fibronectin

BYEOUNG-SOO PARK,[†] JAE-GYU KIM,[†,‡] MI-RAN KIM,[‡] SUNG-EUN LEE,[§] GARY R. TAKEOKA,[#] KI-BONG OH,[†,‡] AND JEONG-HAN KIM*,[†]

School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, South Korea; Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, South Korea; Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, Hawaii 96822; and Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

The inhibitory activity of *Curcuma longa* L. (turmeric) rhizome constituents against sortase A, a bacterial surface protein anchoring transpeptidase, from *Staphylococcus aureus* ATCC 6538p was evaluated. The activity of the isolated compounds (**1**−**4**) was compared to that of the positive control, *p*-hydroxymecuribenzoic acid (pHMB). The biologically active components of *C. longa* rhizome were characterized by spectroscopic analysis as the curcuminoids curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**3**). Curcumin was a potent inhibitor of sortase A, with an IC_{50} value of 13.8 ± 0.7 μg/mL. Bisdemethoxycurcumin (IC_{50} = 31.9 ± 1.2 μg/mL) and demethoxycurcumin (IC_{50} = 23.8 ± 0.6 μg/mL) were more effective than pHMB (IC_{50} = 40.6 ± 1.2 μg/mL). The three isolated compounds (**1**-**3**) showed no growth inhibitory activity against *S. aureus* strain Newman, with minimum inhibitory concentrations (MICs) greater than 200 μg/mL. Curcumin also exhibited potent inhibitory activity against *S. aureus* cell adhesion to fibronectin. The suppression of fibronectin-binding activity by curcumin highlights its potential for the treatment of *S. aureus* infections via inhibition of sortase activity. These results indicate that curcumin is a possible candidate in the development of a bacterial sortase A inhibitor.

KEYWORDS: ***Curcuma longa*** **L.; curcumin;** ***Staphylococcus aureus*****; sortase inhibitory activity; sortase A; antibacterial activity; fibronectin-binding activity; curcuminoids**

INTRODUCTION

The rhizome of turmeric, *Curcuma longa* L. (Zingiberaceae), has been widely used as a yellow coloring agent and spice in many foods. It is also used as an essential ingredient in medicine as a carminative, anthelmintic, laxative, and cure for liver ailment (*1*). The use of turmeric for the antimicrobial activity of its extracts has long been known. Turmeric and its extract have various beneficial effects on human health because they contain a number of monoterpenoids, sesquiterpenoids, and curcuminoids (*2*). Among those secondary metabolites, curcuminoids, such as curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcumin, are yellowish pigments that have antioxidative (*3*), anticarcinogenic (*4*), anti-inflammatory (5), and hypoglycemic effects (*6*).

Gram-positive pathogenic bacteria display surface proteins that play important roles in the adhesion to specific organ tissues, the invasion of host cells, or the evasion of host-immune responses (*7*). These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycan through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases termed sortases (*8*). Two sortase isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in *Staphylococcus aureus* (*9*−*12*). The SrtA isoform plays a critical role in the pathogenesis of Gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesions and other virulence-associated proteins to cell wall peptidoglycan (*11*, *13*). *S. aureus* mutants lacking sortase fail to display surface proteins and are defective in the establishment of infections without affecting microbial viability (*14*, *15*). Therefore, inhibitors of SrtA might consequently be promising candidates for the treatment and/or prevention of Gram-positive bacterial infections.

Currently, there have only been a few papers in the literature describing inhibitors of sortase, due, in part, to the fact that the importance of sortase as a new target has only recently been acknowledged. Initially, Schneewind's group (*16*) showed that methanethiosulfonates or organomercurials displayed inhibitory

* Author to whom correspondence should be addressed (telephone +82-2-880-4644; fax +82-2-873-4415; e-mail kjh2404@snu.ac.kr).
[†] School of Agricultural Biotechnology, Seoul National University.
[‡] Center for Agricultural Biomaterials, Seoul National University.
[§] University of Hawaii.
[#] U.S. Department of Agriculture.

10.1021/jf051765z CCC: $30.25 © 2005 American Chemical Society
Published on Web 10/25/2005

effects on the SrtA reaction. They also observed that vancomycin and moenomycin, inhibitors of cell wall polymerization into peptidoglycan strands, slowed the sorting reaction. However, these antibiotics did not interfere directly with the sorting reaction, but rather changed the physiological concentration of the peptidoglycan precursors. Walker and co-workers replaced the scissile amide bond between the threonine and glycine residues of the LPXTG motif with a diazoketone or chloromethyl ketone group (*17*). Connolly et al. synthesized a peptidyl-vinyl sulfone substrate mimic that irreversibly inhibited SrtA (*18*). Frankel et al. also reported vinyl sulfones as specific SrtA inhibitors (*19*).

Many pharmacological actions of turmeric have been reported to date. However, its inhibitory effect on the Gram-positive SrtA enzyme has not been elucidated. In this study, the active principles isolated from turmeric were characterized by spectroscopic analysis, and their inhibitory effect against SrtA and minimum inhibitory concentrations (MICs) against *S. aureus* (strain Newman) were determined. We also elucidated the potential of curcumin for inhibition of *S. aureus* cell adhesion to fibronectin via fibronectin-binding protein.

MATERIALS AND METHODS

Chemicals. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcumin (all purity >99%) were obtained from Alexis Biochemicals (Kordia, Leiden, The Netherlands) and used as a reference standard. Fluorescent peptide Dabcyl-QALPETGEE-Edans (for SrtA) was synthesized by AnyGen Inc. (Gwangju, Korea). The fibronectin-coated flat-bottom 96-well microtiter plates were obtained from Becton Dickinson Biosciences Discovery Labware (Bedford, MA). *p*-Hydroxymecuribenzoic acid (pHMB) was purchased from Sigma-Aldrich (St. Louis, MO), and all other chemicals were of reagent grade.

Bacterial Strain and Culture Conditions. *Staphylococcus aureus* strain Newman (*20*), a human clinical isolate, represents the wild-type strain in these studies. The isogenic sortase knockout mutants SKM12, sortase A deletion (*srtA*$^-$), and SKM14, sortase A and B deletions (*srtA*$^-$, *srtB*$^-$), were generated by allelic exchanges as previously described (*10*). All strains were obtained from Dr. Olaf Schneewind, University of Chicago, Chicago, IL.

Preparation of Recombinant SrtA. The region of the sortase gene lacking the N-terminal membrane anchor domain was PCR-amplified from the genomic DNA of *S. aureus,* ATCC 6538p, using the primers orf6N-ds-B (5′-AAACCACATATCGATAATTATC-3′) and orf6C-B (5′-TTATTTGACTTCTGTAGCTACAA-3′) (*9*, *12*), and the resulting amplicon was cloned into the TA cloning and expression vector, pBAD/Thio-TOPO (Invitrogen, Groningen, The Netherlands). Competent *Escherichia coli* cells (TOP10, Invitrogen) were then transformed and selected from Luria−Bertani (LB) agar plates containing ampicillin (50 μg/mL). After the selection and verification of positive clones, by dideoxynucleotide DNA sequencing, the transformed bacteria were propagated at 37 °C in LB broth, containing ampicillin (50 μg/mL), and the expression was induced with 0.002% arabinose. Cultures were harvested after a 6-h postinduction incubation at 37 °C, and the recombinant enzyme was purified on a Ni-NTA affinity column, as described previously (*21*).

Isolation and Identification of Compounds. The dried rhizomes (5 kg) of *C. longa* were purchased from a medicinal herb shop, Kyungdong Market (Seoul, South Korea). The rhizomes were finely powdered, extracted with methanol (3 L × 2) at room temperature (24−26 °C) for 2 days, and filtered. The combined filtrate was concentrated under vacuum at 35 °C to yield ∼10%, based on the weight of the dried rhizomes. The MeOH extract (10 g) was fractionated by column chromatography (5.5 × 70 cm) on silica gel (70−230 mesh, Merck, Darmstadt, Germany) using CH_2Cl_2/MeOH (50:1 → 0:100) in a stepwise fashion. The fractions were pooled into five major fractions (fractions M1−M5) on the basis of their TLC profiles. The fraction, M2 (2.91 g), was further separated by column chromatography (4.5 × 65 cm) on silica gel (180 g) using *n*-hexane/Me_2CO (2:1 → 3:2 → 4:3) and CH_2Cl_2/Me_2CO/MeOH (85:1:0 → 0:0:100), yielding orange crystals of curcumin (**1**, 380 mg, R_f = 0.5), orange crystals of demethoxycurcumin (**2**, 79 mg, R_f = 0.35), orange crystals of bisdemethoxycurcumin (**3**, 71 mg, R_f = 0.25), and white crystals of tetrahydrocurcumin (**4**, 38 mg, R_f = 0.6). TLC was performed on precoated silica gel 60 F_{254} and RP-18 F_{254}s plates, which were developed with CH_2Cl_2/Me_2CO (20:1). The plates were sprayed with 10% H_2SO_4 reagent (in EtOH) and heated for detection. The melting points were determined using a Fisher Scientific melting point apparatus and were uncorrected. UV spectra were obtained on a Shimadzu UV−visible spectrophotometer. The NMR spectra were recorded on a Varian Unity Inova 500 spectrometer. The EI-MS (70 eV) spectra were recorded on a JEOL JMS-AX 505H mass spectrometer. The structures of **1**−**4** were identified by comparison of their physical and spectral data with published values (*22*−*24*), respectively, by comparing the spectral data with those reported in the literature. These four compounds were identified on the basis of the following evidence.

*Curcumin (**1**)*: orange crystals; mp 182−184 °C; UV λ_{max} (MeOH) 423 nm; EI-MS, *m/z* 368 $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 7.59 (d, 2H, J = 15.9 Hz, H-1,7), 7.12 (dd, 2H, J = 8.0, 1.9 Hz, H-6′,6″), 7.05 (d, 2H, J = 1.9 Hz, H-2′,2″), 6.93 (d, 2H, J = 8.0 Hz, H-5′,5″), 6.47 (d, 2H, J = 15.3 Hz, H-2,6), 5.87 (br s, 2H, 4′,4″-OH), 5.80* (s, 1H, H-4), 3.95 (s, 6H, 3′,3″-OCH_3) (the asterisk indicates the proton signal assignable to an enol form.); ^{13}C NMR ($CDCl_3$) δ 184.5, 150.0, 148.8, 141.4, 128.2, 123.8, 122.3, 116.2, 111.6, 101.6, 56.3.

*Demethoxycurcumin (**2**)*: orange crystals; mp 168 °C; EI-MS, *m/z* 338 $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 7.61 (d, 1H, J = 15.9 Hz, H-1 or -7), 7.59 (d, 1H, J = 15.9 Hz, H-1 or -7), 7.45 (d, 2H, J = 8.1 Hz, H-2″,6″), 7.12 (dd, 1H, J = 7.8, 1.8 Hz, H-6′), 7.05 (d, 1H, J = 1.8 Hz, H-2′), 6.93 (d, 1H, J = 7.8 Hz, H-5′), 6.88 (d, 2H, J = 8.1 Hz, H-3″,5″), 6.48 (d, 1H, J = 15.9 Hz, H-2 or -6), 6.47 (d, 1H, J = 15.9 Hz, H-2 or -6), 5.86 (br s, 2H, 4′,4″-OH), 5.80 (s, 1H, H-4), 3.95 (s, 3H, 3′-OCH_3); ^{13}C NMR ($CDCl_3$) δ 184.5, 184.4, 160.4, 149.9, 148.7, 141.4, 141.0, 130.9, 128.1, 127.7, 123.8, 122.2, 122.0, 116.8, 116.2, 111.5, 101.7, 56.3.

*Bisdemethoxycurcumin (**3**)*: orange crystals; mp 223−224 °C; EI-MS, *m/z* 308 $[M]^+$; ^{1}H NMR (Me_2CO-d_6) δ 7.61 (d, 2H, J = 15.9 Hz, H-1,7), 7.55 (d, 4H, J = 8.6 Hz, H-2′,6′,2″,6″), 6.89 (d, 4H, J = 8.6 Hz, H-3′,5′,3″,5″), 6.48 (d, 2H, J = 15.9 Hz, H-2,6), 5.97 (s, 1H, H-4), 5.86 (br s, 2H, 4′,4″-OH); ^{13}C NMR (Me_2CO-d_6) δ 184.5, 160.5, 141.0, 130.9, 127.7, 122.0, 116.8, 101.7.

*Tetrahydrocurcumin (**4**)*: white crystals; mp 98−99 °C; ^{1}H NMR ($CDCl_3$) δ 6.82 (d, 2H, J = 7.8 Hz, H-5′,5″), 6.69 (s, 2H, H-2′,2″), 6.65 (d, 2H, J = 7.8 Hz, H-6′,6″), 5.54 (s, 2H, 4′,4″-OH), 5.43 (s, 1H, H-4), 3.85 (s, 6H, 3′,3″-OCH_3), 2.84 (t, 4H, J = 7.6 Hz, H_2-1,7), 2.55 (t, 4H, J = 7.4 Hz, H_2-2,6); ^{13}C NMR ($CDCl_3$) δ 193.2, 146.4, 143.9, 132.5, 120.7, 114.3, 110.9, 99.8, 55.8, 40.3, 31.3.

Sortase Inhibition Assay. Reactions were performed in 300 μL containing 50 mM Tris-HCl, 5 mM $CaCl_2$, 150 mM NaCl, pH 7.5, 55 μg of recombinant $SrtA_{\Delta 24}$, 0.75 g of fluorescent peptide (Dabcyl-QALPETGEE-Edans), and the prescribed concentration of test sample (*10*). Each test compound was dissolved in MeOH and diluted with sterilized distilled water before use (final concentration, 0.5% MeOH, which was found to have no effect on the enzyme activity when the concentration was <1%). Appropriate blanks contained all of the above, with the exception of the test sample. Reactions were carried out for 1 h at 37 °C. Sample fluorescence was measured using emission and excitation wavelengths of 495 and 350 nm, respectively (SpectraMAX Gemini XS, Molecular Devices Co., Sunnyvale, CA). pHMB, a known sortase inhibitor, was used as a positive control (*12*). Experiments were repeated three times independently for each concentration.

Determination of Minimum Inhibitory Concentration (MIC). The MICs of these compounds were determined according to a published protocol (*19*). A culture of *S. aureus* strain Newman (5 mL) in tryptic soy broth (TSB) was grown to saturation at 37 °C and diluted to an OD_{600} of 0.01. The culture was incubated for an additional 2 h and diluted to an OD_{600} of 0.005. In each well of a 96-well plate were mixed 180 μL of cells with 20 μL of a 10 times concentrated test compound solution in 10% DMSO to give a final DMSO concentration of 1%. Culture plates were incubated overnight at 37 °C, and OD_{600} was measured using a Multiskan Spectrum spectrophotometer (Thermo

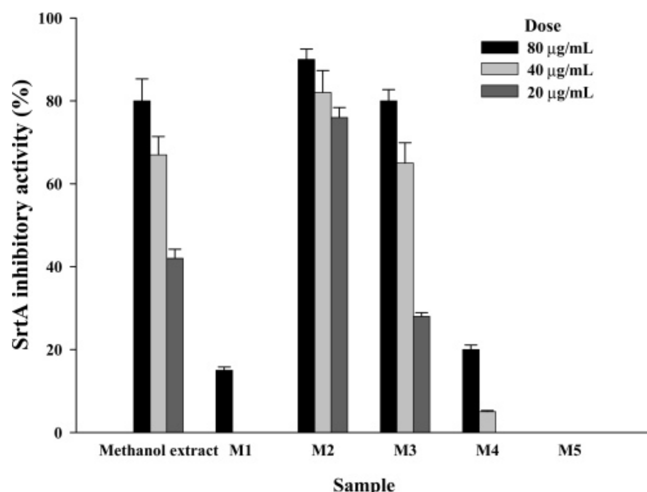

Figure 1. Inhibitory effects of fractions derived from the methanol extract of the dried rhizome of *C. longa* against SrtA from *S. aureus* ATCC 6538p.

Electron Inc., Milford, MA). MIC values were determined as the lowest concentration of test compounds that inhibited cell growth.

Fibronectin Binding Assay. Fibronectin-binding assay was performed as described previously (*25*) with the following modifications. *S. aureus* was grown in TSB at 37 °C to mid log phase (OD_{600} = 0.5). The culture was divided into 5-mL aliquots, and test compound, or control treatment, was added as indicated. Every 30 min for 2.5 h following the addition of test compound, 0.65 mL of cell suspension was removed and pelleted by centrifugation (10000*g* for 10 min). After overnight storage at −20 °C, pellets were resuspended in 0.65 mL of phosphate-buffered saline (PBS) and distributed in 100-μL aliquots to individual wells of fibronectin-coated flat-bottom 96-well microtiter plates. Following 2 h of incubation at 37 °C, the cell suspension was removed, and wells were washed with 0.15 mL of PBS. Bound cells were then fixed by incubation for 30 min with 2% (v/v) glutaraldehyde. Following a second wash with PBS, cells were stained for 15 min with 0.1 mL of crystal violet dye (12.5 g/L). Plates were washed again with PBS, covered with aluminum foil, and allowed to dry overnight (12−16 h). The absorbance at 560 nm was subsequently measured using a microtiter plate reader.

RESULTS AND DISCUSSION

A methanol extract obtained from *C. longa* dried rhizomes possessed inhibitory effect against SrtA, from *S. aureus* ATCC 6538p, with 80, 70, and 42% inhibitory activity at concentrations of 80, 40, and 20 μg/mL, respectively (**Figure 1**). Due to its potent inhibitory activity against SrtA, the methanol extract was separated into five fractions; their activities are given in **Figure 1**. Among these fractions, the M2 fraction had strong SrtA inhibitory activity with a value of 83% at a concentration of 40 μg/mL. The SrtA inhibitory activity-guided fractionation and repeated column chromatography of the MeOH fraction from the dried rhizomes of *C. longa* yielded four curcuminoids, curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and tetrahydrocurcumin (**4**) (**Figure 2**).

The SrtA inhibitory activity of **1**−**4** along with that of a synthetic known SrtA inhibitor, pHMB, was evaluated by comparing the concentration of compounds that causes a ∼50% reduction in SrtA enzyme activity over 1 h [the 50% inhibitory concentration (IC_{50})] (**Table 1**). Among the four compounds tested, **1** exhibited the most potent inhibitory activity against SrtA, with an IC_{50} value of 13.8 ± 0.7 μg/mL. **2** and **3** were less effective than **1**, with IC_{50} values of 23.8 ± 0.6 and 31.9 ± 1.2 μg/mL, respectively. These compounds had stronger activity than pHMB (IC_{50} = 40.6 ± 1.2 μg/mL). **4** did not display any activity against this enzyme.

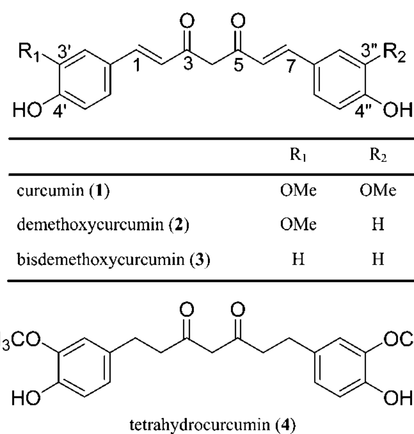

Figure 2. Structures of curcumin and related β-diketone derivatives isolated from *C. longa* L.

Table 1. Inhibitory Effects of Curcuminoids on Sortase A Activity and *S. aureus* Newman Growth

compound	SrtA[a] IC_{50} (μg/mL)	MIC[b] (μg/mL)
curcumin	13.8 ± 0.7	>200
demethoxycurcumin	23.8 ± 0.6	>200
bisdemethoxycurcumin	31.9 ± 1.2	>200
tetrahydroxycurcumin	>200	>200
p-hydroxymecuribenzoic acid (positive control)	40.6 ± 1.2	25

[a] Enzyme inhibitory activities were measured as described in the text. Data represent the mean ± SD (*n* = 3). [b] Average MIC was determined against *S. aureus* strain Newman (*n* = 3).

Sortase inhibitors should act as anti-infective agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability (*14*, *15*). Therefore, we investigated the effect of test compounds on *S. aureus* (strain Newman) cell growth and determined the MICs of these compounds. The antibacterial activity of the test compounds in the MIC assay is shown in **Table 1**. The compounds isolated from *C. longa* rhizome exhibited no growth inhibitory activity (>200 μg/mL) against *S. aureus* strain Newman. However, pHMB, a synthetic known inhibitor of SrtA, showed antibacterial activity with an MIC value of 25 μg/mL.

Cell membrane anchored SrtA catalyzes the covalent anchoring of adhesins such as fibronectin- and fibrinogen-binding proteins that take advantage of the low nanomolar binding affinity for fibronectin or fibrinogen to facilitate attachment to host tissues (*26*). Mutant *S. aureus* strains lacking a functional sortase cannot bind to cell−matrix proteins, such as fibrinogen and fibronectin, and also cannot effect protein A-mediated binding of IgG in vitro (*26*). We thought that sortase inhibitors should inhibit SrtA activity in vivo and in turn reduce fibronectin-binding protein surface display. Thus, we employed an assay (*25*) in which cell adhesion to fibronectin-coated plates was quantified by measuring the absorbance following staining with crystal violet. The capacity of *S. aureus* strain Newman (*srtA*$^+$, srtB$^+$) as well as its isogenic knockout mutants SKM12 (*srtA*$^-$) and SKM14 (*srtA*$^-$, srtB$^-$) to adhere to fibronectin-coated surfaces was investigated. As shown in **Figure 3**, the fibronectin-binding activity of SKM12 and SKM14 was significantly reduced as compared with that of wild type (Newman). The assay also showed the binding activity of the SKM12 mutant was similar to that of SKM14. *S. aureus* SrtA is known to anchor surface proteins involved in adherence to tissue and immune response evasion (*27*, *28*). In a mouse model infection test, the

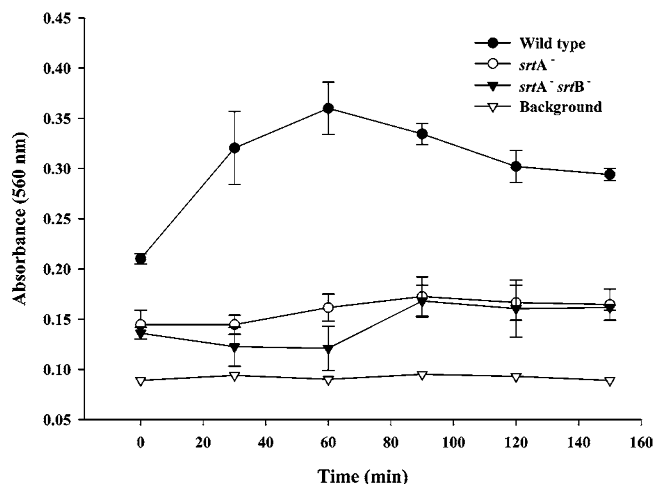

Figure 3. Adhesion of *S. aureus* strain Newman (wild type; *srtA*$^+$, *srtB*$^+$) as well as its isogenic knockout mutants SKM12 (*srtA*$^-$) and SKM14 (*srtA*$^-$, *srtB*$^-$) to fibronectin. The results are the mean values and standard deviations of three replicates.

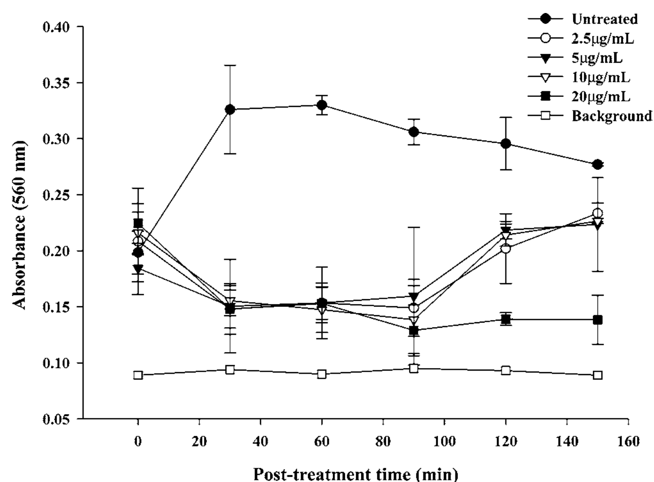

Figure 4. Inhibition of *S. aureus* strain Newman adhesion to fibronectin via fibronectin-binding protein by curcumin. The results are the mean values and standard deviations of three replicates.

srtA mutant *S. aureus* (SKM12) displayed a 2-log reduction in virulence (>99% reduction of bacteria in kidneys), compared with the wild-type strain Newman (*15*). A second area of interest surrounding selected sortase inhibitors was their fibronectin-binding inhibition properties. As expected, treatment of *S. aureus* strain Newman with 1× the SrtA IC_{50} of curcumin significantly reduced the capacity of the bacteria to adhere to fibronectin-coated surfaces (**Figure 4**). The treatment of wild-type strain with curcumin reduced the capacity of the bacterium to adhere to fibronectin-coated surfaces in a dose-dependent manner (2.5−20 μg/mL). Interestingly, a loss of adhesion inhibition was observed after 90 min for samples containing curcumin concentrations below the IC_{50} (13.8 $\pm$ 0.7 μg/mL), whereas 20 μg/mL maintained inhibition through 160 min. These assay data suggest that SrtA inhibitor concentrations above the IC_{50} value are important for the inhibition of *S. aureus* binding to fibronectin via sortase activity. It is important to note that the onset and magnitude of inhibition of fibronectin-binding in *S. aureus* treated with curcumin (20 μg/mL) are comparable to the behavior of untreated sortase deletion strain SKM12 (*srtA*$^-$) as shown in **Figure 3**. This result supported the observation that curcumin was an effective inhibitor of SrtA activity in vivo.

Recently, we found that ethyl acetate fractions obtained from medicinal plant extracts such as *Cocculus trilobus* (rhizome), *Fritillaria verticillata* (tuber), *Liriope platyphylla* (tuber), and *Rhus verniciflua* (bark) exhibited potent inhibitory activity against SrtA (*29*). Additionally, some plant-derived materials have been reported to be active against SrtA, including β-sitosterol-3-*O*-glucopyranoside (IC_{50} = 18.3 μg/mL) from *F. verticillata* (*30*), berberine chloride (IC_{50} = 8.7 μg/mL) from *Coptis chinensis*, and related isoquinoline alkaloids such as beberine sulfate (IC_{50} = 11.5 μg/mL), palmatine chloride (IC_{50} = 12.7 μg/mL), and β-hydrastine (IC_{50} > 80 μg/mL) (*31*). Subsequently, we discovered (*Z*)-3-(2,5-dimethoxyphenyl)-2-(4-methoxyphenyl)acrylonitrile as a novel synthetic small-molecule inhibitor (*32*). The three constituents (**1**−**3**) from turmeric, with IC_{50} values ranging from 14 to 32 μg/mL, have activities that compare favorably to previously reported constituents.

Curcumin, a dietary pigment responsible for the yellow color of curry, has been used as a medicine for the treatment of inflammatory conditions. It has anti-inflammatory and antioxidant activities, and these may contribute to chemopreventive activity (*33*). Furthermore, curcumin derivative studies have recently been done (*34*−*36*). To our knowledge, this is the first report of the inhibition of *S. aureus* adhesion to fibronectin via fibronectin-binding protein by curcumin. It would be important to design specific and powerful inhibitors of this enzyme, which might have very important applications as a new generation of antibiotics.

In conclusion, curcumin isolated from the rhizomes of *C. longa* is active against SrtA in *S. aureus* and has no antibacterial activity against *S. aureus* cell growth. The fibronectin-binding activity data revealed the potential of curcumin for the treatment of *S. aureus* infections via inhibition of sortase activity. The onset and magnitude of inhibition of fibronectin binding in *S. aureus* treated with curcumin are comparable to the behavior of untreated sortase knockout mutants. Further in vivo studies on curcumin are ongoing and will be reported in due course.

LITERATURE CITED

(1) Srimal, R. C. Turmeric: a brief review of medicinal properties. *Fitoterapia* **1997**, *68*, 483−493.

(2) Ammon, H. P.; Wahl, M. A. Pharmacology of *Curcuma longa*. *Planta Med.* **1991**, *57*, 1−7.

(3) Sugiyama, Y.; Kawakishi, S.; Osawa, T. Invovlement of the β-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem. Pharmacol.* **1996**, *52*, 519−525.

(4) Huang, M. T.; Lou, Y. R.; Newmark, H. L.; Reuhi, K. R.; Conney, A. H. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.* **1994**, *54*, 5841−5847.

(5) Hong, J.; Bose, M.; Ju, J.; Ryu, J. H.; Chen, X.; Sang, S.; Lee, M. J.; Yang, C. S. Modulation of arachidonic acid metabolism by curcumin and related β-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. *Carcinogenesis* **2004**, *25*, 1671−1679.

(6) Nishiyama, T.; Mae, T.; Kishida, H.; Tsukagawa, M.; Mimaki, Y.; Kuroda, M.; Sashida, Y.; Takahashi, K.; Kawada, T.; Nakagawa, K.; Kitahara, M. Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. *J. Agric. Food Chem.* **2005**, *53*, 959−963.

(7) Cossart, P.; Jonquières, R. Sortase, a universal target for therapeutic agents against Gram-positive bacteria? *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5013−5015.

(8) Schneewind, O.; Model, P.; Fischetti, V. A. Sorting of protein A to the staphylococcal cell wall. *Cell* **1992**, *70*, 267−281.

(9) Mazmanian, S. K.; Liu, G.; Ton-That, H.; Schneewind, O. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science* **1999**, *285*, 760−763.

(10) Mazmanian, S. K.; Liu, G.; Ton-That, H.; Schneewind, O. An iron-regulated sortase anchors a class of surface protein during *Staphylococcus aureus* pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2293−2298.

(11) Mazmanian, S. K.; Skaar, E. P.; Gaspar, A. H.; Humayun, M.; Gornicki, P.; Jelenska, J.; Joachmiak, A.; Missiakas, D. M.; Schneewind, O. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* **2003**, *299*, 906−909.

(12) Ton-That, H.; Liu, G.; Mazmanian, S. K.; Faull, K. F.; Schneewind, O. Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12424−12429.

(13) Paterson, G. K.; Mitchell, T. J. The biology of Gram-positive sortase enzymes. *Trends Microbiol.* **2004**, *12*, 89−95.

(14) Jonsson, I. M.; Mazmanian, S. K.; Schneewind, O.; Bremell, T.; Tarkowski, A. The role of *Staphylococcus aureus* sortase A and sortase B in murine arthritis. *Microbes Infect.* **2003**, *5*, 775−780.

(15) Mazmanian, S. K.; Liu, G.; Lenoy, E. R.; Schneewind, O. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5510−5515.

(16) Ton-That, H.; Schneewind, O. Anchor structure of staphylococcal surface proteins. IV. Inhibitors of the cell wall sorting. *J. Biol. Chem.* **1999**, *274*, 24316−24320.

(17) Scott, C. J.; McDowell, A.; Martin, S. L.; Lynas, J. F.; Vandenbroeck, K.; Walker, B. Irreversible inhibition of the bacterial cysteine protease-transpeptidase sortase (SrtA) by substrate derived affinity labels. *Biochem. J.* **2002**, *366*, 953−958.

(18) Connolly, K. M.; Smith, B. T.; Pilpa, R.; Ilangovan, U.; Jung, M. E.; Clubb, R. T. Sortase from *Staphylococcus aureus* does not contain a thiolate-imidazolium ion pair in its active site. *J. Biol. Chem.* **2003**, *278*, 34061−34065.

(19) Frankel, B. A.; Bentley, M.; Kruger, R. G.; McCafferty, D. G. Vinyl sulfones: inhibitors of SrtA, a transpeptidase required for cell wall protein anchoring and virulence in *Staphylococcus aureus*. *J. Am. Chem. Soc.* **2004**, *126*, 3404−3405.

(20) Duthie, E. S.; Lorenz, L. L. Staphylococcal coagulase; mode of action and antigenicity. *J. Gen. Microbiol.* **1952**, *6*, 95−107.

(21) Lee, K. Y.; Shin, D. S.; Yoon, J. M.; Kang, H.; Oh, K. B. Expression of a transpeptidase for cell wall sorting reaction, from sortase, *Staphylococcus aureus* ATCC 6538p in *Escherichia coli*. *J. Microbiol. Biotechnol.* **2002**, *12*, 530−533.

(22) Masuda, T.; Isobe, J.; Jitoe, A.; Nakatani, N. Antioxidative curcuminoids from rhizomes of *Curcuma xanthorrhiza*. *Phytochemistry* **1992**, *31*, 3645−3647.

(23) Syu, W.-J.; Shen, C.-C.; Don, M.-J.; Ou, J.-C.; Lee, G.-H.; Sun, C.-M. Cytotoxicity of curcuminoids and some novel compounds for *Curcuma zedoaria*. *J. Nat. Prod.* **1998**, *61*, 1531−1534.

(24) Huang, M.-T.; Ma, W.; Lu, Y.-P.; Chang, R. L.; Fisher, C.; Manchand, P. S.; Newmark, H. L.; Conney, A. H. Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion. *Carcinogenesis* **1995**, *16*, 2493−2497.

(25) Elgalai, I.; Foster, H. A. Comparison of adhesion of wound isolates of *Staphylococcus aureus* to immobilized proteins. *J. Appl. Microbiol*. **2003**, *94*, 413−420.

(26) Alksne, L. E.; Projan, S. J. Bacterial virulence as a target for antimicrobial chemotherapy. *Curr. Opin. Biotechnol.* **2000**, *11*, 625−636.

(27) McDevitt, D.; Francois, P.; Vaudaux, P.; Foster, T. J. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol. Microbiol*. **1994**, *11*, 237−248.

(28) Signas, C.; Raucci, G.; Jonsson, K.; Lindgren, P. E.; Ananthara-maiah, G. M.; Hook, M.; Lindberg, M. Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 699−703.

(29) Kim, S. W.; Chang, I. M.; Oh, K. B. Inhibition of the bacterial surface protein anchoring transpeptidase sortase by medicinal plants. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2751−2754.

(30) Kim, S. H.; Shin, D. S.; Oh, M. N.; Chung, S. C.; Lee, J. S.; Chang, I. M.; Oh, K. B. Inhibition of sortase, a bacterial surface protein anchoring transpeptidase, by β-sitosterol-3-*O*-glucopyranoside from *Fritillaria verticillata*. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2477−2479.

(31) Kim, S. H.; Shin, D. S.; Oh, M. N.; Chung, S. C.; Lee, J. S.; Oh, K. B. Inhibition of the bacterial surface protein anchoring transpeptidase sortase by isoquinoline alkaloids. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 421−424.

(32) Oh, K. B.; Kim, S. H.; Lee, J.; Cho, W. J.; Lee, T.; Kim, S. Discovery of diarylacrylonitriles as a novel series of small molecule sortase A inhibitors. *J. Med. Chem*. **2004**, *47*, 2418−2421.

(33) Satoskar, R. R.; Shah, S. J.; Shenoy S. G., Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1986**, *24*, 651−654.

(34) Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H. K.; Itokawa, H.; Su, C. Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y.; Tsai, M. Y.; Chang, C.; Lee, K. H. Antitumor agents. 217. Curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. *J. Med. Chem.* **2002**, *45*, 5037−5042.

(35) Shim, J. S.; Kim, D. H.; Jung, H. J.; Kim, J. H.; Lim, D.; Lee, S. K.; Kim, K. W.; Ahn, J. W.; Yoo, J. S.; Rho, J. R.; Shin, J.; Kwon, H. J. Hydrazinocurcumin, a novel synthetic curcumin derivative, is a potent inhibitor of endothelial cell proliferation. *Bioorg. Med. Chem.* **2002**, *10*, 2987−2992.

(36) Hahm, E. R.; Gho, Y. S.; Park, S.; Park, C. H.; Kim, K. W.; Yang, C. H. Synthetic curcumin analogs inhibit activator protein-1 transcription and tumor-induced angiogenesis. *Biochem. Biophys. Res. Commun.* **2004**, *321*, 337−344.

Received for review July 21, 2005. Revised manuscript received September 16, 2005. Accepted September 22, 2005. This work was supported by a grant of the Korea Research Foundation Grant funded by the Korean government (MOEHRD) (KRF-2004-005-F00055). This research was partially supported by the Ministry of Education through the Brain Korea 21 Project.

JF051765Z