Candida albicans Mucin Msb2 Is a Broad-Range Protectant against Antimicrobial Peptides

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The human fungal pathogen *Candida albicans* releases a large glycofragment of the Msb2 surface protein (Msb2*) into the growth environment, which protects against the action of human antimicrobial peptides (AMPs) LL-37 and histatin-5. Quantitation of Msb2*/LL-37 interactions by microscale thermophoresis revealed high-affinity binding (dissociation constant [K_D] = 73 nM), which was lost or greatly diminished by lack of O-glycosylation or by Msb2* denaturation. Msb2* also interacted with human α - and β -defensins and protected *C. albicans* against these AMPs. In addition, the lipopeptide antibiotic daptomycin was bound and inactivated by Msb2*, which prevented the killing of bacterial pathogens *Staphylococcus aureus*, *Enterococcus faecalis*, and *Corynebacterium pseudodiphtheriticum*. In coculturings or mixed biofilms of *S. aureus* with *C. albicans* wild-type but not *msb2* mutant strains, the protective effects of Msb2* on the bactericidal action of daptomycin were demonstrated. These results suggest that tight binding of shed Msb2* to AMPs that occurs during bacterial coinfections with *C. albicans* compromises antibacterial therapy by inactivating a relevant reserve antibiotic.

Antimicrobial peptides (AMPs) are major weapons of the human immune system against microbial pathogens, which kill microbes and attract immune cells to the site of infection (1, 2). Both bacteria and fungi are inhibited by AMPs, but they can nevertheless survive if they contain defense mechanisms that limit AMP uptake (3) or inactivate AMPs, e.g., by proteases (4). In addition, microbes may secrete AMP binding proteins as decoys to lower effective AMP concentrations in their vicinity (5–7).

The *Candida albicans* Msb2 membrane protein has been designated a signaling mucin (8) because of its characteristic structure consisting of a short cytoplasmic C terminus, a transmembrane region, and a large, highly O-glycosylated exodomain, which is released in considerable amounts into the growth environment during planktonic and surface growth (9–11). Msb2 fulfills at least two functions to allow *C. albicans* survival and growth in the infected human host. First, external stimuli, including surface growth and cell wall integrity, are signaled via Msb2 to trigger corresponding response or rescue pathways required for growth and hyphal morphogenesis (10, 12–14). Second, we recently found that the secreted Msb2 glycodomain (Msb2*) acts to protect *C. albicans* and *Escherichia coli* against toxicity of AMPs LL-37 and histatin-5 (10). The evidence suggested that Msb2* mediates its protective function by binding of AMPs and that this activity requires intact O-mannosylation by protein O-mannosyltransferases (Pmt proteins). However, binding constants reflecting the affinity of AMPs for Msb2* or any other binding partner have not yet been determined, and the range of AMP substrates bound by Msb2* is still unclear.

Here we report that *C. albicans* Msb2* binds not only to multiple AMPs produced by the human host, including α - and β -defensins (15, 16), but also to daptomycin, an AMP produced by the bacterium *Streptomyces roseosporus*. The lipopeptide antibiotic daptomycin and the glycopeptide antibiotic vancomycin are of special importance in current anti-infectious therapy because these compounds serve as reserve antibiotics for the treatment of multiresistant Gram-positive bacteria (17–19). We demonstrate that the presence of *C. albicans* Msb2* protects the important

bacterial pathogens *Staphylococcus aureus*, *Enterococcus faecalis*, and *Corynebacterium pseudodiphtheriticum* against the inhibitory activity of daptomycin. These results suggest that mixed infections of *S. aureus* and other important bacterial pathogens with *C. albicans* are of particular risk because bacteria are cross-protected by the shed fungal Msb2* protein against peptide antimicrobials, including daptomycin.

MATERIALS AND METHODS

Strains and media. Strains are listed in Table 1. *C. albicans* strain ESCa3 produces a full-length hemagglutinin (HA) and V5 epitope-labeled Msb2 protein. Strain FCCa27 contains a partially deleted allele designated *msb2* Δ 1 (encoding the 406 N-terminal residues of Msb2), which was found to be completely nonfunctional (10). YPD (1% yeast extract, 2% peptone, 2% glucose) or SD (0.67% yeast nitrogen base, 2% glucose) medium was used for growth of *C. albicans* strains on agar or in liquid cultures.

Antimicrobial peptides. The sensitivity of *C. albicans* wild-type strain CAF2-1 to various AMPs was determined as previously described (10) using the AMPs LL-37 (Sigma), histatin-5 (AnaSpec Inc.), human β -defensin-1 (hBD1) (Innovagen), and human α -defensin-1 (hNP-1) (AnaSpec Inc.). Bacteria were tested for their sensitivity against daptomycin (Sigma) or vancomycin (Hikma Pharmaceuticals PLC).

Proteins. Strain ESCa3 produces full-length Msb2 doubly epitope tagged in its large extracellular and small cytoplasmic domains (HA and V5 epitopes, respectively); strain ESCa25 encodes Msb2 variant Msb2- Δ N, which lacks residues 33 to 481 of Msb2 (10). The extracellular domains of both Msb2 proteins (functional in AMP binding) were almost quantitatively released into the growth medium and are referred to as Msb2* and Msb2 Δ N*, respectively. The secreted HA-tagged Msb2* or

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TABLE 1 Strains

Strain	Genotype	Reference
<i>C. albicans</i>		
CAI4	<i>ura3Δ::imm434/ura3Δ::imm434</i>	27
CAF2-1	<i>ura3Δ::imm434/URA3</i>	27
FCCa27	As CAI4 but <i>msb2Δ1::hisG/msb2Δ1::hisG-URA3-hisG</i>	10
ESCa3	As CAI4 but <i>msb2Δ1::hisG/msb2Δ1::hisG LEU2/LEU2::pES11a (ACT1p-MSB2^{HA-V5})</i>	10
ESCa18	As CAI4 but <i>pmt1Δ::hisG/pmt1Δ::hisG LEU2/LEU2::pES11a (ACT1p-MSB2^{HA-V5})</i>	10
ESCa25	As CAI4 but <i>msb2Δ1::hisG/msb2Δ1::hisG LEU2/LEU2::pES14 (ACT1p-MSB2-ΔN^{HA-V5})</i>	10
Bacteria		
<i>S. aureus</i> ATCC 35556 (Sa113)	As NCTC 8325 but restriction deficient	30
<i>E. faecalis</i> ATCC 29212	Wild type	31
<i>C. pseudodiphtheriticum</i> ATCC 10701	Wild type	32
<i>B. subtilis</i> ATCC 6051	Wild type	29

Msb2-ΔN* protein fragments were purified by affinity chromatography and quantified immunologically, as previously described (10). Mucin from porcine stomach (type III, 0.5 to 1.5% bound sialic acid, partially purified powder; Sigma) was solubilized in water and autoclaved (20); the solubilized mucin was centrifuged (4,000 rpm, 30 min), and the supernatant was used in assays. Mucin concentrations were calculated assuming a molecular weight of 2,000,000 (21).

MST. Microscale thermophoresis (MST) analysis was performed using a NanoTemper Monolith NT.115 apparatus (22, 23). A constant amount of 94 nM carboxyfluorescein-tagged LL-37 (5-FAM-LL-37; AnaSpec, Fremont, CA) was incubated for 30 min at 37°C in the dark with different concentrations of Msb2 protein in HEPES-KOH (pH 7.4 or 8) or morpholineethanesulfonic acid (MES) (pH 6) buffer containing 0.005% SDS. Then, 10 μl of the samples was loaded into standard glass capillaries (Monolith NT Capillaries) and thermophoresis analysis was performed (settings for the light-emitting diode and infrared [IR] laser were 80% and 50%, respectively). In a competition experiment, 4.25 μM unlabeled LL-37 or 50 μM daptomycin was preincubated for 15 min at 37°C with the samples before addition of FAM-LL-37.

Antimicrobial peptide assay. *C. albicans* was grown in YPD medium at 30°C to an optical density at 600 nm (OD₆₀₀) of 0.3. Cells were harvested by centrifugation and washed with and resuspended in phosphate-buffered saline (PBS). Triplicate assays contained 5 μl cell suspension and different concentrations of AMPs in a total volume of 25 μl. Assay mixtures were incubated for 1.5 h at 37°C, diluted 500-fold, and plated on YPD agar. CFU were determined after 2 days of growth at 30°C. Bacteria were grown and diluted in LB (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride) or Loeffler medium (0.25% peptone, 0.15% beef extract, 0.125% sodium chloride, 0.5% dextrose) containing horse serum (Sigma) at 37°C to an OD₅₉₅ of 0.3. Cells were harvested by centrifugation, washed with PBS, and resuspended in growth medium. Triplicate assay mixtures of different concentration of antibiotics in a total volume of 1 ml were incubated for 30 min (10 μg/ml daptomycin) and 4 h (4 μg/ml vancomycin) at 37°C, diluted 10,000-fold, and plated on LB or Loeffler agar. CFU were determined after 1 day of growth at 37°C. In the case of hBD1, this assay was carried out in a hypoxic chamber (0.2% O₂, 0.2% CO₂) because hBD1 is activated in this environment (24).

AMP coculturing assay. *C. albicans* strains (CAF2-1, FCCa27, and ESCa3) were grown in 5 ml YPD medium to an OD₆₀₀ of 8. *S. aureus* (Sa113) cells (3 × 10⁷/ml) were added, and incubation was continued for 30 min at 37°C. Daptomycin (5 μg/ml) was added, and *S. aureus* cells (CFU) were determined by plating on LB agar containing caspofungin (100 ng/ml) at different times of coinoculation in the presence or absence of daptomycin.

AMP biofilm assay. *C. albicans* was pregrown in YPD and *S. aureus* was pregrown in LB medium overnight at 37°C. Cells were harvested by centrifugation and resuspended in PBS; *C. albicans* cellular aggregates

were then disassembled in a bath sonifier (10 min), pelleted, and resuspended again in PBS. Numbers of cells were estimated by measuring the optical density at 600 nm (*C. albicans*) or 595 nm (*S. aureus*) using a standard curve, and cells were resuspended in RPMI 1640 medium at a concentration of 10⁶ cells per ml. Strains were inoculated, separately or in combination, at a concentration of 10⁶ cells per ml into wells of 24-well polystyrene cell culture plates, and cells were allowed to form homogeneous or mixed biofilms by growth in RPMI 1640 medium at 37°C for 24 h or 48 h. Biofilms were treated for 5 h with daptomycin (10 μg/ml) or vancomycin (4 μg/ml) at 37°C before removal of biofilms using a sterile scalpel. Biofilms were disassembled in RPMI 1640 medium by strong vortexing, diluted, and plated on LB plates containing caspofungin (100 ng/ml) to prevent fungal growth. *S. aureus* CFU were determined after 24 h at 37°C. Experiments were repeated three times with each strain. The statistical significance of differences between groups of data was analyzed using an unpaired *t* test calculating two-tailed *P* values (GraphPad Prism 5 program).

RESULTS

MST reveals high-affinity interaction of Msb2* and LL-37. Microscale thermophoresis (MST) is an effective method to assess dissociation constants for the interaction of a fluorescent ligand with its unlabeled binding molecule in solution (22, 23). In this method, the avidity of binding is typically demonstrated by the rate of disappearance of the fluorescent signal from a zone of heating, a process termed “thermophoresis.” We used MST technology to precisely quantitate binding of the human cathelicidin LL-37 to the secreted N-terminal fragment of the Msb2 surface protein of the fungal pathogen *C. albicans*. In previous experiments, it had been shown that this Msb2 fragment (referred to as Msb2*) is released during fungal growth into the environment and is able to neutralize the toxic effects of LL-37 on *C. albicans* and *E. coli* (10).

A series of binding assays with a constant amount of 5-FAM-LL-37 and various concentrations of affinity-purified Msb2* showed concentration-dependent thermophoresis reaching a half-maximal rate at 73 nM, which corresponds to the dissociation constant (*K_D*) of the binding reaction (Fig. 1A and B). This high binding affinity was retained in an Msb2 variant lacking 450 residues in the N-terminal domain (Msb2-ΔN*) (Table 2), consistent with AMP inactivation functions of the deleted variant (10). Furthermore, binding of Msb2* to LL-37 was not affected greatly between pH 6 and 8. On the other hand, binding was abolished by heat inactivation of Msb2* and also by preincubation of Msb2* with unlabeled LL-37 (Table 2), demonstrating that LL-37 bind-

FIG 1 Microscale thermophoresis assay to quantitate Msb2/LL-37 interaction. (A) 5-FAM-LL-37 (94 nM) and Msb2* were allowed to interact and heated locally to generate a temperature gradient; the rate of fluorescence disappearance as a function of Msb2* concentration was recorded. Results are from a representative set of assays done in triplicate; concentrations are indicated by colored dots, and arrows indicate the begin and end of heating. (B) The degree of thermophoresis was plotted against Msb2* concentration and yielded a half-maximal rate at 73 nM. Mean values of three measurements $\pm$ standard deviations are shown.

ing occurs on a specific site of Msb2 in its native protein conformation. Interestingly, *in vitro* binding of 5-FAM-LL-37 was also highly dependent on *O*-glycosylation of Msb2*, since binding was lost using an Msb2* preparation from a *pmt1* mutant host strain (ESCa18). This finding is in agreement with lowered AMP inactivation by the *pmt1* mutant (10).

Human cells produce several mucins, which reside on cellular surfaces and in the intercellular space of tissues (25). Human mucins, like the *C. albicans* Msb2 mucin, are highly *O*-glycosylated proteins, which conceptually could compete for the binding of AMPs. As a model for human mucin, we assayed binding of 5-FAM-LL-37 to porcine gastric mucin, which is structurally similar to human mucin (20, 26). MST measurements revealed a much lower affinity of binding to porcine mucin ($K_D = 876$ nM) than to *C. albicans* Msb2* (Table 2). Thus, it appears that unlike mammalian mucin, Msb2* exhibits a remarkable affinity for the cathelicidin LL-37.

Msb2* protects against different AMPs, including the anti-biotic daptomycin. In previous studies, we reported that the se-

creted glycofragment of the signaling mucin Msb2 (Msb2*) protects *C. albicans* and *E. coli* against the toxicity of human AMPs LL-37 and histatin-5 (10). It is shown here that Msb2* also prevents toxicity of two other human AMPs, human α -defensin-1 (hNP-1) and human β -defensin-1 (hBD1) (Table 3). This Msb2* activity was demonstrated by exposing *C. albicans* CAF2-1 (27) for 1.5 h to AMPs in the absence or presence of purified Msb2* (10 μ g), followed by assessment of fungal viability by determination of CFU. For all tested AMPs, a clear protective effect of Msb2* against *C. albicans* killing was observed. Note that in the assay for hBD1 activity, the incubation was carried out not only under normoxia but also in an hypoxic chamber (0.2% O₂, 0.2% CO₂), because under hypoxia, reduction of disulfide bonds leads to activation of this AMP (24). We indeed observed increased killing by hBD1 under hypoxia compared to normoxia, but importantly, Msb2* provided protection under both conditions.

AMPs not only are of human origin but are also produced by numerous microorganisms (28). Some microbial AMPs, including daptomycin and vancomycin, are of special importance for antibiotic therapy of infectious diseases, because they serve as reserve antibiotics for the treatment of multiresistant Gram-positive bacteria (17–19). Here we tested if the presence of *C. albicans* Msb2* affects the antibiotic activity of daptomycin, and we could

TABLE 2 Dissociation constants for binding of 5-FAM-LL-37

Protein	pH	K_D (nM, mean $\pm$ SD)
Msb2*	7.4	73.1 $\pm$ 23.3
	6.0	70.7 $\pm$ 14.7
	8.0	86.1 $\pm$ 17.9
Msb2* + unlabeled LL-37 ^a	7.4	328 $\pm$ 46.9
Msb2* boiled ^b	7.4	418 $\pm$ 33.7
Msb2 Δ N*	7.4	86.4 $\pm$ 17.6
	6.0	58.1 $\pm$ 17.5
	8.0	55.3 $\pm$ 6.34
Msb2* from <i>pmt1</i> Δ	7.4	951 $\pm$ 80.9
Mucin (porcine stomach)	7.4	876 $\pm$ 48.9
Msb2* + daptomycin ^c	7.4	498 $\pm$ 16.8

^a Preincubation for 15 min with 4.25 μ M unlabeled LL-37.

^b 95°C, 15 min.

^c Preincubation for 15 min with 50 μ M daptomycin.

TABLE 3 Msb2* protects *C. albicans* against different AMPs

Antimicrobial peptide and condition	Net charge	Concn (μ M)	CFU (%), mean $\pm$ SD	
			Without Msb2*	With Msb2*
LL-37	+6	26.7	51.7 $\pm$ 0.7	97.5 $\pm$ 0.9
Histatin-5	+12	26.4	79.6 $\pm$ 2.1	114.6 $\pm$ 3.7
hNP-1	+3	23.2	72.7 $\pm$ 4.4	98.8 $\pm$ 6.7
hBD1				
Normoxia	+6	37.2	82.1 $\pm$ 7.8	96.3 $\pm$ 1.6
Hypoxia ^a	+6	37.2	75.4 $\pm$ 2.0	107.1 $\pm$ 4.0

^a 0.2% O₂, 0.2% CO₂.

FIG 2 Msb2 protects bacterial pathogens against daptomycin. Bacteria were incubated with 10 µg/ml daptomycin for 30 min in the absence (black bars) or presence (white bars) of Msb2* (50 µg/ml). Numbers of surviving bacterial cells were determined by plating (CFU counts), with values for a control incubation without daptomycin set as 100%. Mean values of three measurements $\pm$ standard deviation are shown. ***, $P < 0.0001$. The bacterial strains were *S. aureus* Sa113, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6051, and *C. pseudodiphtheriticum* ATCC 10701.

indeed show that Msb2* protected the Gram-positive bacteria *Bacillus subtilis* (29), *S. aureus* (30), *E. faecalis* (31), and *C. pseudodiphtheriticum* (32) against the inhibitory action of daptomycin (Fig. 2). Furthermore, daptomycin competed with 5-FAM-LL37 for binding to Msb2* (Table 2), suggesting binding of daptomycin to a specific site on the Msb2* protein, which also binds LL-37. In additional experiments we also tested Msb2* for its protective effects against the glycopeptide vancomycin, but we did not observe significant protection by Msb2* using this antibiotic (data not shown).

Collectively, these results suggest that the shed *C. albicans* Msb2 glycofragment protects *C. albicans* and several Gram-positive bacteria, including important human bacterial pathogens, against a wide range of human AMPs and the relevant current antibiotic daptomycin.

Cross-species protection mediated by Msb2 in coculturings.

The Gram-positive bacterium *S. aureus* and the fungus *C. albicans* are currently among the leading nosocomial pathogens, often coinfecting patients and leading to high mortality (33). It is known that *C. albicans* and *S. aureus* are able to form complex polymicrobial biofilms (34), in which *S. aureus* is protected from the action of vancomycin (35). We considered the possibility that Msb2* produced by live *C. albicans* cells could also help to protect cohabitating cells of *S. aureus* against daptomycin. In a first series of experiments, mixed planktonic *C. albicans*-*S. aureus* cultures were generated by adding bacteria to stationary cultures of *C. albicans* strains either producing or not producing Msb2*. In the absence of daptomycin, bacterial growth occurred equally with all *C. albicans* strains (Fig. 3A, left panel). However, in the presence of daptomycin, killing of *S. aureus* was maximal in cohabitation with the *msb2* mutant (strain FCCa27), which is unable to produce functional Msb2, while cohabitation with Msb2*-producing strains led to significantly reduced killing of bacteria (Fig. 3A, right panel). This finding was confirmed in a second experimental approach using cohabitation in mixed biofilms. In triplicate experiments, Msb2-producing strains of *C. albicans* (CAF2-1 and ESCa3) or the *msb2* mutant (strain FCCa27) were allowed to form a mixed biofilm with *S. aureus* (Sa 113) for 24 or 48 h at 37°C in

FIG 3 Cocultured *C. albicans* protects *S. aureus* against daptomycin. (A) Planktonic coculturing. *C. albicans* strains CAF2-1 (wild type [wt]), FCCa27 (*msb2*Δ1), and ESCa3 (*msb2*Δ1 [MSB2]) were grown in 5 ml YPD medium to an OD₆₀₀ of 8. *S. aureus* (Sa113) cells (3×10^7 /ml) were added, and incubation was continued for 30 min at 37°C. No daptomycin (left panel) or 5 µg/ml daptomycin (right panel) was added, and numbers of viable *S. aureus* (Sa113) cells (CFU) were determined by plating on LB-caspofungin agar at different times of coincubation. (B) Biofilm coculturing. Mixed biofilms of *C. albicans* and *S. aureus* were allowed to form for 24 or 48 h before addition of daptomycin (10 µg/ml). Biofilms were disassembled following 24 h (black bars) or 48 h (white bars), and numbers of bacterial survivors were determined by plating on LB-caspofungin agar. CFU of a single-species *S. aureus* biofilm without added daptomycin were set as 100%.

RPMI 1640 medium; in a control experiment, *C. albicans* cells were omitted to allow growth of an *S. aureus* monoculture biofilm. Following growth, 10 µg/ml daptomycin was added and cells were incubated further for 5 h. Biofilms were then disassembled, and live cell counts were determined on LB agar containing caspofungin to select for bacterial survivors.

The results indicate that *S. aureus* growth was inhibited to the same extent by daptomycin in the control culture and in cohabitation with the *msb2* mutant, while significantly greater numbers of *S. aureus* survivors were found in the coculture with the *C. albicans* Msb2* strain (Fig. 3B). It is concluded that production of Msb2* by *C. albicans* can partially overcome daptomycin toxicity and protect cohabitating *S. aureus* cells.

DISCUSSION

Disease caused by *C. albicans* manifests itself in the form of massive fungal growth on skin and mucous layers, as well as at multi-

ple systemic body sites. Approximately one-fourth of all *C. albicans* bloodstream infections appear to be polymicrobial (33), and coinfection with *C. albicans* and the important bacterial pathogen *S. aureus* in mice is synergistic to increase mortality (36). *C. albicans* and *S. aureus* were shown to interact directly by forming a mixed biofilm (34) that depended to a considerable extent on the *C. albicans* Als3 cell wall protein (37). Importantly, mixed biofilm formation with *C. albicans* enhanced the resistance of *S. aureus* to vancomycin (35), which, like the more recent antibiotic daptomycin, is being used as a reserve-type antibiotic to combat multiresistant Gram-positive bacterial pathogens, including *Staphylococcus*, *Enterococcus*, and *Corynebacterium* species. Here we describe a novel molecular strategy explaining how the *C. albicans*-*S. aureus* cohabitation can improve survival of the bacterial pathogen during antibiotic treatment. It is shown that a fragment of the Msb2 surface protein, which is secreted in considerable amounts during planktonic and surface growth of *C. albicans* (10), is able to protect *S. aureus*, *E. faecalis*, and *C. pseudodiphtheriticum* against the toxic activity of daptomycin. This protection was detected using purified Msb2* protein but also in coculturing with Msb2*-producing *C. albicans* strains and is expected to occur by binding of daptomycin to Msb2*, because daptomycin competed with LL-37 in MST measurements. The Msb2* binding activity lowers the effective concentration of antibiotic in liquid culture and around mixed microbial colonies and biofilms occurring in the host. Thus, these data indicate that for treatment of *S. aureus* infections, attention should be given to *C. albicans* coinfection, which should be treated simultaneously to ensure the success of antibacterial antibiotic therapy.

Here we have quantitated for the first time the affinity of a fungal secretory protein for a human AMP by using MST technology (22, 23). This method allows measurement of the affinity of protein-protein interactions in solution, which more closely reflects native conditions than binding assays using surface-bound molecules. The average dissociation constant (K_D) of 73 nM indicates a very high affinity of the cathelicidin LL-37 for the Msb2* glycoprotein, whereas the abundant glycoprotein mucin, on the other hand, binds to LL-37 only weakly (K_D = 876 nM). Thus, mammalian mucins and possibly other human glycostructures (38) may loosely bind or store AMPs, but they lack tight AMP binding activity (like *C. albicans* mucin Msb2*), because such activity could prevent release and accessibility of human AMPs, which would be counterproductive during microbial attack. MST technology may be used in the future to determine dissociation constants of AMPs with other body or microbial glycostructures (5–7, 38–42).

The structural requirements leading to tight binding of AMPs to Msb2* remain to be determined in detail. We previously determined that AMP interactions still occurred with an Msb2* variant lacking 40% of the N-terminal residues (variant Msb2- Δ N*); furthermore, the presence of O-mannosyl residues (43) was found to be important for efficient AMP regulation (10). Here we verified by MST technology the importance of O-mannosylation for AMP binding by demonstrating that Msb2* isolated from a *pmt1* mutant showed low affinity for LL-37. Denatured Msb2*, however, had minor AMP binding activity, indicating that glycostructures *per se* are not sufficient for binding. Regarding the types of AMPs bound by Msb2*, a surprising variety of structures was found, including the human AMPs LL-37, histatin-5 (10), and α - and β -defensins, as well as the microbial AMP daptomycin. Net

charges of AMPs bound by Msb2* vary greatly and range from -1 (daptomycin) to $+12$ (histatin-5), indicating that charge interactions may contribute to but are not essential for these interactions. Interestingly, binding of LL-37 to Msb2* was competed by daptomycin, suggesting one or more specific binding sites for various AMPs on Msb2*. It will be of great interest to clarify the molecular details of these interactions and to establish why some peptides, including human AMPs and daptomycin, bind strongly to Msb2* while other peptides, including vancomycin, interact only weakly.

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