Characterization of Histatin 5 with Respect to Amphipathicity, Hydrophobicity, and Effects on Cell and Mitochondrial Membrane Integrity Excludes a Candidacidal Mechanism of Pore Formation*

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From the ‡Department of Periodontology and Oral Biology, Boston University Goldman School of Dental Medicine, Boston, Massachusetts 02118, the ¶Department of Oral Biochemistry, Academic Center for Dentistry (ACTA), 1081 BT, Amsterdam, The Netherlands, and the †Department of Food Technology and Nutritional Sciences, Laboratory of Food Microbiology, 6703 HD, Wageningen, The Netherlands

Histatin 5 is a 24-residue peptide from human saliva with antifungal properties. We recently demonstrated that histatin 5 translocates across the yeast membrane and targets to the mitochondria, suggesting an unusual antifungal mechanism (Helmerhorst, E. J., Breeuwer, P., van’t Hof, W., Walgreen-Weterings, E., Oomen, L. C. J. M., Veerman, E. C. I., Nieuw Amerongen, A. V., and Abee, T. (1999) J. Biol. Chem. 274, 7286–7291). The present study used specifically designed synthetic analogs of histatin 5 to elucidate the role of peptide amphipathicity, hydrophobicity, and the propensity to adopt α-helical structures in relation to membrane permeabilization and fungicidal activity. Studies included circular dichroism measurements, evaluation of the effects on the cytoplasmic transmembrane potential and on the respiration of isolated mitochondria, and analysis of the peptide hydrophobicity/amphipathicity relationship (Eisenberg, D. (1984) Annu. Rev. Biochem. 53, 595–623). The 14-residue synthetic peptides used were dh-5, comprising the functional domain of histatin 5, and dhvar1 and dhvar4, both designed to maximize amphipathic characteristics. The results obtained show that the amphipathic analogs exhibited a high fungicidal activity, a high propensity to form an α-helix, dissipated the cytoplasmic transmembrane potential, and uncoupled the respiration of isolated mitochondria, similar to the pore-forming peptide PGLa (Peptide with N-terminal Glycine and C-terminal Leucine-amide). In contrast, histatin 5 and dh-5 showed fewer or none of these features. The difference in these functional characteristics between histatin 5 and dh-5 on the one hand and dhvar1 and dhvar4, and PGLa on the other hand correlated well with their predicted affinity for membranes based on hydrophobicity/amphipathicity analysis. These data indicate that the salivary protein histatin 5 exerts its antifungal function through a mechanism other than pore formation.

Throughout living nature, distinct groups of cationic peptides have been identified that display antimicrobial activity in vitro. Best characterized with respect to their structure-function relationship are magainins from the skin of the frog Xenopus laevis (1, 2), cecropins from the giant silk moth Hyalophora cecropia (3), and defensins (α and β) from human leukocytes and various epithelial sources, respectively (4–6). These peptides show considerable variation in chain length, hydrophobicity, and charge distribution; however, they share the common feature of being cationic in nature and able to adopt 70 ordered amphipathic α-helix or β-sheet conformations in structure-promoting solvents, such as trifluoroethanol, and in membrane-mimicking liposome vesicles. Their antimicrobial mode of action is believed to arise from the attraction to negatively charged surface molecules on the target cell and the subsequent formation of a membrane-spanning porelike structure, thereby altering membrane permeability leading to cell lysis.

The regulated and constitutive expression of such cationic peptides provides an immediate protection to tissues that are continuously subjected to microbial challenges (7, 8). In the oral cavity, the hard and soft tissues are constantly exposed to a variety of microorganisms that can lead to caries and periodontal disease. This condition requires that antimicrobial components are continually secreted to provide a permanent line of defense against microbial invasion or at least to maintain the harmonious relationship between the commensal microflora and the host (9). Examples of such salivary antimicrobial systems are histatins. Histatins are a group of histidine-rich cationic peptides that are secreted by the parotid and the submandibular/sublingual human salivary glands (10, 11). At least 12 fragments have been identified (12) that derive from the proteolytic degradation of two gene products, histatin 1 and histatin 3 (13). Histatin 5 has been shown to be the most potent residues (14, 15). Despite a great interest in understanding the mechanism of histatin function, the molecular events leading to cell death have so far been largely elusive (16–20).

Salivary histatins differ from other natural antimicrobial peptides in a number of respects. First, histatins are enriched in the amino acid histidine. Histidine has an isoelectric point of 6.5 and, therefore, modulates the cationicity of the peptide considerably at lower pH values. In addition to this, histidine side chains are known participants in metal chelation. Indeed, histatin 5 is able to form complexes with various metal ions (21), and this adds potential biological properties to this peptide compared with other basic peptides. Secondly, although

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histatins like other antimicrobial peptides adopt helical conformations in hydrophobic environments (15, 22), the amphipathicity of this helix quantified by the hydrophobic moment is rather low compared with other antimicrobial peptides, such as the magainins (16). Because peptide amphipathicity is believed to be a key factor in governing the cytolytic activity of pore-forming basic antimicrobial peptides (23), it is questionable whether histatin 5 operates by the same mode of action. We recently demonstrated that histatin 5 translocates across the yeast membrane and shows intracellular targeting to the mitochondria, suggesting an unusual antifungal mechanism (18). The present investigation focuses on physical peptide parameters, such as amphipathicity, hydrophobicity, and the propensity to adopt helices in relation to these unique functional characteristics of histatin 5. The biophysical and biological results obtained show that histatin 5, in contrast to more amphipathic peptides employed for comparison, is not a classic pore former, and these aspects are discussed with respect to its capacity to translocate across the yeast membrane.

**EXPERIMENTAL PROCEDURES**

**Antimicrobial Peptides**—Histatin 5, the C-terminal domain dh-5, and peptides derived from this domain, dhvar1 and dhvar4, were chemically synthesized as previously described (16, 24). Synthetic PGLa (Peptide with N-terminal Glycine and C-terminal Leucine-amide) was a kind gift from H. V. Westerhoff (Dept. of Molecular Cell Physiology, Vrije Universiteit, Amsterdam).

**Circular Dichroism**—CD spectra were recorded under computer control on an AVIV 62DS CD spectropolarimeter (AVIV Associates, Inc., Lakewood, NJ) from 250 to 183 nm at 25 ± 0.5 °C using a 0.1-cm quartz cuvette. Peptides were dissolved in pure water, in pure trifluoroethanol (TFE), or in a mixture of both solvents to a final concentration of 0.04–0.14 mg/ml. Spectra (1-nm intervals, 1 st interval with 15–15 repeats) were averaged and corrected for base-line contribution. Molar ellipticity values [θ] were calculated according to Equation 1,

\[
[\theta] = \frac{\theta \times MW}{c \times l \times d} \times 10 \times 10^3 \quad \text{(Eq. 1)}
\]

where \( \theta \) is the displacement from the base-line value for the full range in degrees, MW is the mean residue weight of the amino acids in the protein structure, \( l \) is the path length of the cuvette in cm, and \( c \) is the concentration of the protein in g/ml.

**Antifungal Assay**—C. albicans 315 (ATCC 10231) was cultured from a glycerol stock on Sabouraud dextrose broth (Difco), washed once in 1.2 M sorbitol (Sigma), and suspended in 1.2 M sorbitol supplemented with 50 mM potassium phosphate buffer, pH 7.4. For every milliliter of yeast suspension with an \( A_{600} \) of 10.2 μl of β-mercaptoethanol (Sigma) and 10 units of yeast lytic enzyme (ICN Biomedicals, Costa Mesa, CA) were added, and the suspension was incubated for 1 h at 37 °C. Spheroplasts were collected by centrifugation in a Sorvall tabletop centrifuge with a H-1000B rotor at 2100 × g for 4°C, washed four times in 1.2 M sorbitol, suspended in 5–10 ml of 1.2 M sorbitol, and stored at 4 °C for up to 16 h. After centrifugation for 5 min at 2100 × g, spheroplasts were suspended in 15 ml of 0.4 M sorbitol, 0.2% (w/v) bovine serum albumin (fraction V, Sigma), and 10 mM imidazole (Fisher), pH 6.4, and homogenized on ice for 10 min using a manual Potter-Elvehjem homogenizer. The homogenate was mixed 1:1 with buffer containing 1 M sorbitol, 25 mM KH₂PO₄, 4 mM EGTA (Sigma), 0.2% (w/v) bovine serum albumin, and 10 mM imidazole, pH 6.4, and centrifuged in a Sorvall RC-5B centrifuge with a SS-34 rotor for 5 min at 10000 × g at 4 °C. The supernatant was carefully removed and centrifuged using the same rotor for 10 min at 12000 × g at 4 °C. The reddish pellet containing the mitochondria was suspended in a small volume (typically 1 ml) of 0.6 M mannitol, 2 mM EGTA, 0.2% bovine serum albumin, and 10 mM imidazole, pH 6.4, and kept on ice. In each experiment the same volume of the mitochondrial suspension was used for polarographic measurements (final \( A_{600} \) of 0.15–0.25)

**Measurement of Mitochondrial Respiratory Activity**—Mitochondrial oxygen consumption was measured using a biological oxygen monitor model 5300 equipped with a 5331 standard oxygen probe (YSI, Inc., Yellow Springs, OH). Measurements were performed in air-saturated buffer containing 0.65 mM mannitol, 2 mM MgCl₂, 16 mM KH₂PO₄, 10 mM imidazole, pH 6.4. State 2 or basal rate respiration was measured after the addition of NADH (Sigma) to a final concentration of 1 mM, a substrate that can be directly oxidized by yeast mitochondria (28). State 3 respiration was determined after the addition of ADP (Sigma) to a final concentration of 0.33 mM. State 4 respiration was determined after all the ADP was used and the respiration had returned to the basal rate.

**RESULTS**

**Structural Analysis**—The amino acid sequences, the isoelectric point, the mean hydrophobicity (H), and the mean hydrophobic moment (μ) of histatin 5, the fungicial domain dh-5, the two substitution analogs dhvar1 and dhvar4, and the two magainin peptides, magainin 2 and PGLa, are summarized in Table I. The design of dhvar1 and dhvar4 was based on a helical wheel projection of dh-5 (16). The peptides are all basic with isoelectric points ranging from 10 to 12. Major differences were observed in the mean hydrophobicities and mean hydrophobic moments. Histatin 5 and dh-5 have a relatively low hydrophobic moment and, therefore, are weakly amphipathic in contrast to dhvar1, dhvar4, magainin 2, and PGLa, which have a high hydrophobic moment and are highly amphipathic. These amphipathicities were calculated assuming pure helix conformation. Previous studies with histatin 5 and a number of recombinant histatin analogs have shown that these peptides all adopted helical conformations in pure TFE (29, 30). To assess the inducibility of α-helix formation and to detect potential differences between histatin 5, dh-5, dhvar1, and dhvar4, the CD spectra were recorded in aqueous solvents with a stepwise increase of the TFE content (Fig. 1). In pure water (water/TFE ratio of 100/0), the spectra of all peptides showed a maximum molar ellipticity between 215 and 220 nm and a minimum molar ellipticity below 200 nm, which is typical for random coil conformation. At increasing TFE content of the solvent, α-helical conformations were inducible in all peptides, characterized by two negative CD bands between 218–222 and 206–208 nm and a maximum molar ellipticity below 200 nm. The peptides differed in their propensity to adopt helix conformations. Dh-5 was most reluctant to adopt helices being only helical in pure TFE, whereas dhvar4 most rapidly adopted helices being only random coil in pure water. Histatin 5 and
Histatin 5 showed the spectra consistent with mixtures of random coil and \( \alpha \)-helix conformations in mixtures of water and TFE. Although the tendency to adopt helices at increasing hydrophobicity was predominant, it is interesting to note that it was repeatedly found that peptide histatin 5 did not assume the highest degree of \( \alpha \)-helix in pure TFE but did in a 50/50 water/TFE mixture.

| Peptide  | Sequence                  | Charge at pH 7.0 | PI  | \( H^b \) | \( \mu^c \) |
|----------|---------------------------|------------------|-----|----------|----------|
| Histatin 5 | D S H A K R H H G Y K R K F H E K H H S H R G Y | 5+  | 10.3 | -0.704  | 0.377    |
| dh-5     | K R K F H E K H H S H R G Y | 4+  | 10.5 | -0.847  | 0.294    |
| dhvar1   | K R L F K E L K F S L R K Y | 5+  | 10.6 | -0.293  | 0.710    |
| dhvar4   | K R L F K K L L F S L R K Y | 6+  | 11.2 | -0.125  | 0.726    |
| Magainin 2 | G I G K F L H S A K K F G K A F V G E I M N S | 3+  | 10.0 | -0.180  | 0.689    |
| PGLa     | G M A S K A G A I A G K I A K V A L K A La | 5+  | 12.0 | 0.231   | 0.600    |

\( ^{a}\) The net peptide charge and the isoelectric point (PI) were calculated using the software program “Peptool.” In PGLa, the positive charge at the N terminus was not compensated by a negative charge at the C terminus, thereby increasing the net peptide charge by 1 unit.

\( ^{b} H \), mean hydrophobicity of the most amphipathic 11-residue sequence calculated from the sum of the normalized consensus hydrophobicity values (36). In histatin 5, this sequence started from Ala\(^{4}\). In dh-5, dhvar1, and dhvar4, this sequence started from Arg\(^{2}\). In magainin 2, this sequence started from Lys\(^{4}\), and in PGLa, this sequence started from Glu\(^{11}\).

\( ^{c} \mu \), mean hydrophobic moment of the most amphipathic 11-residue sequence in the peptide in pure helix conformation.

Kinetics of Killing of C. albicans—To compare the kinetics of the killing of C. albicans, cells were exposed to histatin 5, dh-5, dhvar1, and dhvar4 for different time intervals after which the viability of the cells was determined in a colony-forming assay (Fig. 2). At 8.3 \( \mu \)M, histatin 5, dhvar1, and dhvar4 caused a 100% reduction in the viability of the yeast inoculum, which was equivalent to a reduction by 3 log units in viable counts.
Histatin 5, almost 100% reduction in viability was observed after 20 min of incubation. Incubation for 90 min with 8.3, 3.3, 1.6, and 0.8 μM histatin 5 resulted in a 100, 90, 35, and 15% reduction in viable counts, respectively.

With dh-5 comprising residues 11–24 of histatin 5, very comparable results were obtained. On the other hand, exposure of the cells to only 1.6 μM dhvar1 or only 0.8 μM dhvar4 resulted in a 100% reduction in viable counts within 6 min of incubation, demonstrating the higher activity of these analogs.

It is well known that the presence of ions affects the antimicrobial activity of several antimicrobial peptides including histatins (31). Sensitivity studies to ions with histatin 5, dh-5, dhvar1, and dhvar4 were carried out with equally effective rather than equimolar concentrations to compensate for the higher molar potencies of dhvar1 and dhvar4 compared with histatin 5 and dh-5. Such concentrations were derived from experiments as shown in Fig. 2. Under these conditions, histatin 5, dhvar1, and dhvar4 showed comparable dependence on the ionic strength (Table II). Because the peptides are well soluble in all buffers used, these data suggest that ions interfere with the peptide-cell interaction, either by preventing electrostatic interactions or by modifying groups on the yeast cell envelope.

Uncoupling of the Yeast Cytoplasmic Transmembrane Potential—Although the initial interaction for histatin 5, dh-5, dhvar1, and dhvar4 is presumably electrostatic in nature, the ability of these peptides to interact with lipophilic portions of the bilayer and to insert into the microbial membrane might be significantly different. Such interactions, which may lead to the disruption of membrane integrity, have often been studied using liposome vesicles as a model system for microbiological membranes (32, 33). In the present study, the effect of peptide on aspects of the integrity of whole C. albicans cells was studied by the measurement of changes in the cytoplasmic membrane potential. For this purpose, diS-C₃(5), a fluorescent dye with membrane potential-dependent distributional properties, was used. This probe is fluorescent in solution but autoquenches when it accumulates intracellularly (26). It should be pointed out that the accumulation of the dye is driven by the potential across the cytoplasmic membrane rather than the potential across the mitochondrial membrane because sodium azide, which dissipates the mitochondrial transmembrane potential (18), does not prevent the intracellular accumulation of diS-C₃(5) (data not shown). For this experiment, relatively high doses of peptide were chosen so that the dissipating effect could be determined immediately after the addition of the peptide. Fig. 3 shows that the exposure of C. albicans cells, loaded with diS-C₃(5), to 18 μM dhvar4 results in an increase in the fluorescence signal to the level just after adding the probe to the cells, indicating the complete release of the dye into the extracellular environment due to cell membrane permeation. At this concentration, histatin 5 and dh-5 had no effect, and testing at a 6-fold higher concentration to compensate for their lower molar potency only caused a small increase in fluorescence intensity.
Uncoupling of Respiration in Isolated Yeast Mitochondria—

Mitochondria were isolated from *C. albicans* for two purposes. The first purpose was to investigate the effect of histatin 5 on the respiratory activity of isolated yeast mitochondria. Interest in this stems from our previous observation that fluorescein isothiocyanate-labeled histatin 5 associates with *C. albicans* mitochondria *in situ* (18). The second purpose was to assess whether dhvar4, which was shown in the previous experiment to alter the membrane integrity in whole cells, would in analogy of magainin peptides (34, 35) act as an uncoupler of the respiration of isolated mitochondria. In Fig. 4A, the respiratory activity of the mitochondria is shown in the presence of the substrate NADH (State 2 respiration), after the addition of ADP (State 3), and after the depletion of ADP (State 4). The respiratory control ratio defined as the ratio of the respiratory rates in States 3 and 4 was 2.6, indicating that the respiration was coupled to ATP formation. The increase in respiration upon the addition of ADP is a measure for the integrity of the mitochondrial inner membrane, which was a prerequisite to study the uncoupling potential of the peptides. The peptides were added to mitochondria respiring in State 2. Histatin 5 at a final concentration of 33 μM inhibited mitochondrial respiration by 63%. Inhibition of the respiratory chain was verified by the observation that the addition of the uncoupler CCCP after histatin 5 did not increase respiration. dh-5 at the same concentration had no inhibitory effect on respiration, and as expected, the addition of CCCP after dh-5 increased respiration. In contrast to histatin 5 and dh-5, dhvar4 increased mitochondrial respiration by a factor of 2.2 ± 0.3 at a concentration of 3.6 μM. For dhvar1 similar results were obtained (data not shown), indicating that both dhvar1 and dhvar4 act as uncouplers. For comparison, CCCP at a concentration of 33 μM increased respiration by a factor of 2.7 ± 0.5 (Fig. 4B). PGLα was even more active than CCCP because at a concentration of 3.4 μM, a respiratory increase of 3.7 ± 0.5 was found, which is similar to its previously reported effect on isolated rat liver mitochondria (34).

The Coordinates of the Peptides in a Hydrophobicity Plot—

Attempts to define the hydrophobic properties of a helix have been made using the hydrophobicity plot developed by Eisenberg (36). In such a plot the vertical axis represents the mean hydrophobic moment (μ), and the horizontal axis represents the mean hydrophobicity (H) of a given peptide sequence (36). Empirical data have allowed the assignment of plot domains representing “surface-seeking,” “globular,” and “transmembrane” protein characteristics. For each peptide, the mean hydrophobicity and the hydrophobic moment were calculated for the most amphipathic 11-residue sequence spanning three helical turns using the normalized consensus hydrophobicity scale (36) (Table I) and were plotted as shown in Fig. 5. The coordinates calculated for histatin 5 and dh-5 correspond to the values of protein sequences predominantly found in “globular” protein regions, which do not show an affinity for membranes. In contrast, the coordinates of dhvar1, dhvar4, magainin 2, and PGLα correspond to the values of protein sequences found in association with membrane surfaces, and such proteins have therefore been labeled as “surface-seeking.” The latter region is hypothesized to contain sequences with an affinity for a hydrophobic/hydrophilic interphase.

**DISCUSSION**

The results presented in this manuscript show that there is a clear difference in the biological activities exerted by histatin 5 and dh-5 on the one hand and two amphipathic analogs dhvar1 and dhvar4 on the other hand. These differences include their effect on cell viability and membrane integrity. Comparison of these biological activities with physical peptide parameters “mean peptide hydrophobicity” (H) and “mean peptide hydrophobic moment” (μ) show that the biological properties of the peptides used in this study relate well to their...
The antifungal potencies of histatin 5, dh-5, dhvar1, and dhvar4, magainin 2, and PGLa. Each coordinate (H, μ) represents the mean hydrophobicity (H) and the mean amphipathicity (μ) of the most amphipathic 11-residue segment in the peptide as calculated in Table I.

coordinates (H, μ) in a hydrophobicity plot. The peptides with coordinates that fall within the region that predicts an affinity for membranes display an interaction with membranes leading to permeabilization, as measured by the dissipation of the cytoplasmic transmembrane potential in C. albicans cells, and the uncoupling of respiration in isolated mitochondria. This shows that the combination of H and μ is a key determinant for these properties, and it even suggests that this plot can be used as an approach to predict the potential lytic activity of basic antimicrobial peptides in biological systems. Histatin 5, of which the coordinates fall outside the surface-seeking region in the hydrophobicity plot, showed no such permeabilizing activity either in whole cells or in isolated mitochondria. This indicates that histatin 5 cannot be considered a classical pore former such as the magainins.

It should be emphasized that for the calculation of peptide amphipathicity it was assumed that the peptide was in α-helical conformation. Our CD results showed that helices were inducible in all peptides, but there was a tendency that these conformations were more rapidly induced in dhvar1 and dhvar4, the peptides which are biologically more active. As anticipated, analysis of amphipathicity of the peptides as a function of their predicted structure revealed that conversion into an α-helical conformation is required for optimizing their amphipathic characteristics. Because the absolute requirement for peptide helical conformations to confer biological activity has been questioned (37) including that for histatins (38), we speculate that not helix formation per se but rather the ability of peptides to rapidly adopt an amphipathic structure may be of importance. The data obtained with the peptides employed in this study show that membrane activity is determined by both the amphipathicity of this helix and the hydrophobicity properties of the peptide.

The antifungal potencies of histatin 5, dh-5, dhvar1, and dhvar4 were compared in kinetic experiments using a colonymaking assay to determine cell viability. In this assay, a 48-h incubation time separates the kinetic experiment from the actual determination of cell viability. This commonly used assay is potentially flawed by the possible continuous effect of adsorbed or internalized peptide. That this was not the case is evidenced by the fact that 6-min time intervals of peptide (e.g., histatin 5) exposure resulted in distinct viability differences. Any residual peptide activity would have obscured such differences. Furthermore, in a previous study we simultaneously used a direct cell killing dye exclusion method and the indirect plating technique and found a perfect correlation between both assays (18). In the present study, we used the plating technique and found a clear distinction between the functional characteristics of the natively occurring sequences of histatin 5 and dh-5 and the non-native, amphipathic sequences of dhvar1 and dhvar4. First, on a molar basis, natural histatin 5 is less efficient and less fast in killing C. albicans than its amphipathic variants. Second, although the initial interactions of histatin 5, dh-5, and the designed amphipathic analogs are inhibited by salt, there is a remarkable difference in their ultimate effect on membrane integrity. It is interesting to note that even at high concentrations, histatin 5 and dh-5 displayed only a small dissipating effect on the transmembrane potential. Other studies showed no effect of histatin 5 on the cytoplasmic transmembrane potential of C. albicans using the related fluorescent probe, 3,3′-dipentyloxacarbocyanine iodide (DiOC5(3)) (19). A slight disturbance of the transmembrane potential would be expected because exposure of yeast cells to histatin 5 has been shown to promote the influx of propidium iodide (18) and the efflux of ATP and other components absorbing at 260 nm (19, 39). These observations indicate that histatin 5 must alter yeast membrane integrity to a certain extent in a nonspecific way. The present data obtained with whole cells together with the inability of histatin 5 to uncouple the respiration of isolated mitochondria, however, strongly argue against a classical pore model for membrane permeabilization.

Unlike histatin 5, dhvar4 causes an immediate dissipation of the cytoplasmic transmembrane potential upon addition to the cells, indicating complete membrane permeabilization consistent with the formation of membrane-spanning pore-like structures as described previously for magainins (32, 34, 40). Another interesting observation is that dhvar4, similar to PGLa, has an uncoupling effect on isolated mitochondria. Such activity indicates that respiration is uncoupled from ATP production due to the dissipation of the proton gradient across the mitochondrial inner membrane, whereas the functionality of the respiratory chain is completely maintained. This supports a mechanistic model for dhvars and magainins to form membrane-spanning peptide clusters that coexist with a functional respiratory chain. The permeabilizing effect assessed here against both cytoplasmic and mitochondrial membranes is likely to account for the acute cell death that occurs upon exposure of whole yeast cells to these kinds of amphipathic peptides.

It has now been well established that histatin 5 associates with the yeast cytoplasmic membrane and is subsequently internalized (18, 20). A similar observation has recently been reported for the 32-residue histatin 3 peptide from which the 24-residue histatin 5 peptide is derived (41). It has been established that histatin 5 is relatively reluctant to adopt helical structures and that its helices are only weakly amphipathic. One might speculate that these features of histatins, which are different from typical amphipathic pore-forming peptides, prevent histatins from becoming entrapped within the membrane and facilitate their ability to enter the cytoplasm. It has furthermore become clear that the ultimate events leading to cell death are more complex than hitherto thought based on the fact that the killing of C. albicans by histatins requires active cell metabolism (18, 41). This indicates that the cell itself may be actively involved in its own demise and supports a mechanistic model for killing by histatins that is not restricted to a direct membrane effect.

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FIG. 5. Hydrophobicity plot of histatin 5, dh-5, dhvar1, dhvar4, magainin 2, and PGLa. Each coordinate (H, μ) represents the mean hydrophobicity (H) and the mean amphipathicity (μ) of the most amphipathic 11-residue segment in the peptide as calculated in Table I.
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The first author’s name was misspelled. The correct spelling is shown above.

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Blue light sensing in higher plants.

John M. Christie and Winslow R. Briggs

Page 11460: Ref. 33 is incorrect. Please substitute the following two references for Ref. 33:

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