Salivary Histatin 5: Dependence of Sequence, Chain Length, and Helical Conformation for Candidacidal Activity*

(Received for publication, October 11, 1989)

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Histatin 5 (Asp1'-Ser-His-Ala4'-Lys-Arg-His1'-Gly-Tyr-Lys-Arg12'-Lys-Phe-His-Glu18-Lys-His-His19-His-Arg-Gly-Tyr24), one of the basic histidine-rich peptides present in human parotid saliva and several of its fragments, 1–16 (N16), 9–24 (C16), 11–24 (C14), 13–24 (C12), 15–24 (C10), and 7–16 (M10), were synthesized by solid-phase procedures. Native histatin 5 from human parotid saliva was also purified. Their antifungal activities on two strains of Candida albicans have been studied and their conformational preferences both in aqueous and non-aqueous solutions examined by circular dichroism. The synthetic histatin 5, C16, and C14 peptides were highly active and inhibited the growth of C. albicans. The candidacidal activity data of synthetic histatin 5 were comparable to the values of the native histatin 5 isolated from parotid saliva and those reported previously, although the assay system used and the strains examined were different. The C16 fragment was as active as the whole peptide itself, whereas the N16 fragment was far less active than C14, suggesting that the sequence at the C-terminal is important for its candidacidal activity. An increase in the chain length of the C-terminal sequence from 12 to 16 residues increased the candidacidal activity, thereby indicating that a peptide chain length of at least 12 residues is necessary to elicit optimum biological activity. The CD spectra of these linear peptides showed that they are structurally more flexible, and they adopt different conformations depending on the solvent environment. CD studies provided evidence that histatin 5 and the longer fragments, C16, N16, and C14 preferred α-helical conformations in non-aqueous solvents such as trifluoroethanol and methanol, while in water and pH 7.4 phosphate buffers, they favored random coil structures. The shorter sequences seemed to adopt either turn structures or unordered structures both in aqueous and non-aqueous solutions. It appears that the sequence at the C-terminal of histatin 5 with a minimum chain length of 14 residues and an α-helical conformation are the important structural requirements for appreciable candidacidal activity.
Synthesis, CD, and Structure-Function Analyses of Salivary Histatin 5 and Fragments

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Candidacidal Activity—The efficacy of native and synthetic histatin 5 and its fragments to induce loss of viability of C. albicans was measured at various concentrations and the results are summarized in Fig. 2 and Table I. These data provided in Fig. 2 and Table I are for the DIS strain. C. albicans strain 28366 was found to be 10–20% more susceptible to the fungicidal activity of histatins. Both synthetic and native histatin 5 and the C-terminal fragments C16 and C14 were found to be highly active in inducing loss of viability of C. albicans (Fig. 2). The percent loss of viability of C. albicans induced by the salivary histatin 5 and synthetic histatin 5 at varying concentrations was found to be almost identical. The activity data of synthetic histatin 5 (Fig. 2 and Table I) are also comparable to those reported for native histatin 5 (Oppenheim et al., 1988), even though the strains of C. albicans examined and the assay system used are different. The C16 fragment, which constitutes the C-terminal sequence of histatin 5, was as active as histatin 5 itself (Fig. 2b). At a concentration of 50 μM, histatin 5, C16, and C14 induced ~95% loss of viability of C. albicans (Fig. 2a), whereas N16 and C12 exhibited only ~60 and ~50% activity, respectively (Table I). The N16 and C12 fragments were less active (Fig. 2 and Table I). The shorter C terminal fragment C10 was weakly active (35%) at a concentration of 300 μM, while the middle sequence M10 showed only ~20% activity (Fig. 2b). The C16 fragment, which constitutes the C-terminal sequence of histatin 5, was as active as histatin 5 itself (Fig. 2b). At a concentration of 50 μM, histatin 5, C16, and C14 induced ~95% loss of viability of C. albicans (Fig. 2a), whereas N16 and C12 exhibited only ~60 and ~50% activity, respectively (Table I). The N16 and C12 fragments were less active (Fig. 2 and Table I). The shorter C terminal fragment C10 was weakly active (35%) at a concentration of 300 μM, while the middle sequence M10 showed only ~20% activity (Fig. 2b).

The percent loss of viability of C. albicans induced by the C-terminal fragments of histatin 5 as a function of chain length at three different concentrations is shown in Fig. 2c.

TABLE I

Loss of viability of Candida albicans (DIS) induced by histatins

<table>
<thead>
<tr>
<th>Peptide conc.</th>
<th>Native histatin 5</th>
<th>Synthetic histatin 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>0.05</td>
<td>98 ± 1</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>0.10</td>
<td>96 ± 2</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>0.50</td>
<td>94 ± 3</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>1.00</td>
<td>92 ± 4</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>2.00</td>
<td>90 ± 5</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>5.00</td>
<td>88 ± 6</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>10.00</td>
<td>86 ± 7</td>
<td>90 ± 5</td>
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<tr>
<td>20.00</td>
<td>84 ± 8</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>50.00</td>
<td>82 ± 9</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>100.00</td>
<td>80 ± 10</td>
<td>84 ± 5</td>
</tr>
</tbody>
</table>

*Expressed as [1-(cell survival after histatin incubation)/(cell survival in buffer alone)] × 100.

Fig. 1. Amino acid sequences of natural histatins and synthetic fragments of histatin 5.

Fig. 2. Dependence of percent loss of viability of C. albicans (DIS) on peptide concentration. a, native histatin 5 and the C-terminal fragments. b, synthetic histatin 5, N16, C16, and M10. c, dependence of percent loss of viability of C. albicans (DIS) on peptide chain length of the C-terminal fragments at 25, 50, and 100 μM concentrations. Values represent duplicate analyses.

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FIG. 2. Dependence of percent loss of viability of C. albicans (DIS) on peptide concentration. a, native histatin 5 and the C-terminal fragments. b, synthetic histatin 5, N16, C16, and M10. c, dependence of percent loss of viability of C. albicans (DIS) on peptide chain length of the C-terminal fragments at 25, 50, and 100 μM concentrations. Values represent duplicate analyses.
An excellent correlation exists between the chain length of the C-terminal fragments and their candidacidal activity at different concentrations. The activity was zero for Cl0 which has the C-terminal 10 amino acid residues of histatin 5. The activity was significantly increased to ~40–50% (Fig. 2c) when the chain length was increased to 12 residues as in the case of C12 which has the additional Lys-Phe-segment at the N-terminal of Cl0. The activity was further enhanced to ~80–90% (Fig. 2e) for the 14-residue peptide C14 having an additional Lys-Arg-segment at the N-terminal of C12. The 16-residue fragment C16 restored the activity of the parent peptide itself. Linking of the tetrapeptide segment Lys-Arg-Phe- to the N-terminal of C10 increases its basic character as well as the activity of C14, thereby suggesting that these residues at positions 11, 12, 13, and 14 in histatin 5 may be functionally important for its fungicidal properties. However, the fragments M10 and N16 which have these 4 residues in their sequences were far less active. This indicates that these residues at the N termini, as in the case of C16 and C14, increase the activity. Increasing the chain length to an appropriate size may give rise to a favorable conformational feature of the peptide backbone which results in better interaction of the peptide with C. albicans. An increase in chain length has been reported to favor helical conformation and enhanced biological activity in peptide antibiotics of microbial origin (Raj et al., 1988).

There are seven histidines, four lysines, and three arginines in the parent 24 residue histatin 5. The high biological activity of the C16 fragment suggests that the four histidines at position 15, 18, 19, and 21, the three lysines at 11, 13, and 17 and the two arginines at 12 and 22 in histatin 5 may be functionally important for candidacidal activity. The diminished activity of N16 and the enhanced activity of the C16 suggest that Asp and Ser residues present at the N-terminal of histatin 1, 3, and 5 may not be essential for candidacidal activity. The activity studies of histatin 5 and its fragments thus indicate that the sequence at the C-terminal of histatin 5 and a chain length of 14 residues may be the important requirements for candidacidal activity.

**Circular Dichroism Studies**—The CD spectra of histatin 5 and its fragments in trifluoroethanol, methanol, water, and pH 7.4 phosphate buffer solutions were recorded between 180 and 250 nm and the CD parameters are summarized in Tables II–IV. The CD spectra of synthetic histatin 5 in trifluoroethanol and water, pH 7.2, are in good agreement with those of the native histatin 5 (Fig. 3). In TFE and methanol, histatin 5, C16, C14, and N16 showed two strong negative bands at 216–220 and 206–208 nm and a strong positive band at 190–195 nm (Figs. 3, 4 and Table II), characteristic of helical structures (Jung et al., 1975). Histatin 5 exhibited a positive band at ~194 nm ([θ]M = +91.49 × 10⁴ deg cm² dmol⁻¹) and two negative bands at 208 nm ([θ]M = -46.66 × 10⁴ deg cm² dmol⁻¹) and at 220 nm ([θ]M = -46.66 × 10⁴ deg cm² dmol⁻¹) in TFE (Table II). Similar CD bands were observed for histatin 5 in methanol (Fig. 3). The fragments of C16, C14, and N16 also exhibit similar band shapes in TFE and methanol (Table II). The two strong negative bands at 206–208 nm and at 216–220 nm observed for these peptides in TFE (Fig. 4) correspond to the long wavelength component of exciton split of the π-π* transition and the n-π* transition, respectively (Jung et al., 1975). These CD spectra are characteristic of helical polypeptides and such CD band shapes have been generally ascribed to α-helical structures (Greenfield and Fasman, 1969; Jung et al., 1975; Mayr et al., 1979; Oekonomopoulos and Jung, 1980; Sudha et al., 1983). The CD band parameters for the various peptides in TFE and meth-
TABLE IV

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Trifluoroethanol</th>
<th>Methanol</th>
<th>Water</th>
<th>Phosphate buffer (pH 7.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histatin 5</td>
<td>α-Helix</td>
<td>α-Helix</td>
<td>Random coil</td>
<td>Random coil</td>
</tr>
<tr>
<td>N16 (1-16)</td>
<td>α-Helix</td>
<td>α-Helix</td>
<td>Random coil</td>
<td>Random coil</td>
</tr>
<tr>
<td>C16 (9-24)</td>
<td>α-Helix</td>
<td>α-Helix</td>
<td>Random coil</td>
<td>Random coil</td>
</tr>
<tr>
<td>C14 (11-24)</td>
<td>α- and β10-Helix</td>
<td>α- and β10-Helix</td>
<td>Random coil</td>
<td>Random coil</td>
</tr>
<tr>
<td>C12 (13-24)</td>
<td>Helix &amp; turn</td>
<td>β-Turns</td>
<td>β-Turns</td>
<td>β-Turns</td>
</tr>
<tr>
<td>C10 (15-24)</td>
<td>β-Turns</td>
<td>β-Turns</td>
<td>β-Turns</td>
<td>β-Turns</td>
</tr>
<tr>
<td>M10 (7-16)</td>
<td>β-Turns</td>
<td>β-Turns</td>
<td>Random coil</td>
<td>Random coil</td>
</tr>
</tbody>
</table>

**Fig. 3.** CD spectra of synthetic histatin 5 in TFE (a), native histatin 5 in TFE (b), synthetic histatin 5 in methanol (c), synthetic histatin 5 in water (d), native histatin 5 in water (e) and synthetic histatin 5 in pH 7.4 sodium phosphate buffer (f).

**Fig. 4.** CD spectra of N16 (a), C16 (b), C14 (c), C12 (d), C10 (e), and M10 (f) in TFE.

anal and the conformational inferences drawn from CD data are provided in Table II and IV, respectively. The ratio of the intensities of the two bands, \( R = \Theta_{\alpha-\omega}/\Theta_{\alpha-\omega} \), has often been taken as an index of helical structures, with values of \( \sim 1 \) being observed for α-helical polypeptides (Greenfield and Fasman, 1969). It is clear from the data in Table II that \( R \) values for histatin 5, and the 16 residue fragments C16 and N16 are \( \sim 0.9 \) in TFE and methanol, suggesting that they prefer a largely α-helical conformation in these non-aqueous solvents (Table IV). There was a marked decrease in the molar ellipticities of the CD bands observed for C16, N16, and C14, when compared with those observed for histatin 5 (Table II). Such decreases in ellipticities may be due to chain length effects in helical peptides (Vijayakumar et al., 1984). The diminished ellipticity values observed for the fragments therefore reflect the shorter chain length of these fragments.
The use of absolute ellipticity values to estimate helix content in peptides of low molecular mass, by comparison with polypeptide standards (>100 kDa) has been reported to be a "hazardous exercise," generally leading to considerable underestimation of the helix content (Jung et al., 1975; Mayr et al., 1979; Ockonomopoulos and Jung, 1980; Sudha et al., 1983).

The R values for the fragment Cl4 were 0.77 and 0.78 in TFE and methanol, respectively (Table II). A decreased R value of -0.78 has been observed for stereochemically constrained helical peptides which favor 310-helical conformations (Sudha et al., 1983) and for the peptide antibiotic alamethicin which adopts a largely α-helical conformation with a short 310-helical portion (Jung et al., 1975; Fox and Richards, 1982). Conformational flexibility of the peptide backbone has also been suggested as a probable reason for decreased R values observed for helical peptides (Sudha et al., 1983). The low R values observed for Cl4 in TFE and methanol may be due to an α-helical conformation with a portion of 310-helix in these solvents or due to the presence of populations of α- and 310-helical conformations (Table IV). The CD spectrum of Cl2 in TFE (Fig. 4d) does not fully resemble the CD spectra of helical peptides. However, the band due to the exciton split of the K-K* transition was observed at -208 nm. The R value of 0.65 for this sample indicates a highly distorted helical structure. It also exhibited a negative band at -188 nm. The spectrum appears to be a composite spectrum of P-turn and helical conformations. In methanol, Cl2 showed two bands at 194 nm ([θ]_194 = -0.98 × 10^4 deg cm^2 dmol^-1) and at 218 nm ([θ]_218 = +2.29 × 10^4 deg cm^2 dmol^-1) (Table II), characteristic of C' CD spectra for Type II β or open reverse turn structure (Smith and Pease, 1980). The CD spectra of C10 both in methanol and TFE exhibited a positive band at -216–218 nm (Table III) and a negative band at -190–196 nm, resembling the C' spectra for reverse turn structures. The CD spectra of the middle fragment M10 in TFE and methanol (Fig. 4d and Table II) are similar to the CD spectra reported for the pentapeptides YPNDV and YPIDV in water (Dyson et al., 1988). These spectra have been interpreted in terms of small populations of β-turns in solution (Table IV).

In water and in sodium phosphate buffer solution (pH =
7.4), histatin 5 and the longer fragments, C16, N16, and C14 exhibited a weak positive band around 218-224 nm and a strong negative band at ~190-195 nm (Figs. 3, 5 and Table III). The CD spectra of these peptides in aqueous solvents are characteristic of random coil structures observed for polypeptides (Greenfield and Fasman, 1969). The ordered helical structures observed for these peptides in hydrophobic solvents are disrupted by breaking the intramolecular interaction between CO and NH groups of peptides by the competitive interaction of a highly polar hydrogen bonding solvent (water). The CD spectra of the shorter fragments, C12, C10, and M10 in water and phosphate buffer solution showed a positive band at 220-224 nm and a negative band at 195-206 nm, which can be ascribed to populations of different β-turn conformations (Table IV). The CD studies showed that the regular helical conformations observed for histatin 5 and the longer fragments C16, N16 and C14 in non-aqueous weakly hydrogen bonding solvents are disrupted by the strongly hydrogen bonding water, suggesting the structural flexibility of this class of peptides. The intramolecular hydrogen bonds between the NH of the fifth residue and the CO of the first residue (5 → 1), which stabilize α-helical conformations (Fig. 6) and those between the NH of the fourth residue and CO of the first residue (4 → 1), which stabilize 310-helical conformations (Fig. 6) in non-aqueous solutions are disrupted by an aqueous environment which leads to random coil structures.

**Conformation and Candidacidal Activity**—The structural flexibility of histatin 5 and its active fragments as observed from CD studies makes it difficult to unambiguously correlate the conformation and biological activity of these linear peptides. However, the candidacidal activity data and the (1) results show some evidence that helical conformation may, indeed, be important for their antifungal properties. The shorter fragments, C10 and M10, had the least tendency to adopt helical conformation in any solvent due to their short chain length, and they were found to be correspondingly least active (Fig. 2, a and b). When the chain length was increased from 10 to 12 residues, as in the case of C12 which shows a weak tendency to form helical conformation in TFE, there was an increase in candidacidal activity to 48% at a concentration of 50 μM (Fig. 2). The fragment C14 with a chain length of 14 residues had a greater tendency to adopt a helical conformation (77-78%) both in TFE and methanol, and it regained 94% candidacidal activity (Table I). The C-terminal fragment C16 with its longer chain length had an overwhelming tendency to prefer largely α-helical structure in hydrophobic solvents and it possessed almost all the activity of the parent peptide, histatin 5 (Table I). Thus, there exists an excellent correlation between chain length, tendency to form helical conformation and candidacidal activity of histatin 5 and its fragments. However, the N-terminal N16 peptide was far less active than C16 although it favored α-helical conformation in non-aqueous solvents, indicating that the candidacidal activity is also dependent on the C-terminal sequence.

Peptide antibiotics of microbial origin such as alamethicin, suzukacin, and emerininc which possess high percentages of α-aminoisobutyric acid exhibit antibacterial and antifungal properties. They are structurally rigid due to the presence of the stereochemically constrained α-aminoisobutyric acid and adopt helical conformations (Mueller and Rudin, 1968; Argoudelis and Johnson, 1974; Ponday et al., 1977; Mathew and Balaram, 1983; Bosch et al., 1985). They have been reported to alter membrane permeability by forming channes across membranes (Jung et al., 1981). The growth inhibitory effects of histatins on C. albicans have been reported to be concomitant with loss of potassium from yeast cells, thereby suggesting that these peptides may also alter membrane permeability (Pollock et al., 1984; Oppenheim, 1989). The candidacidal activities of histatin 5 and the longer fragments which possess high tendencies to favor helical conformations in a hydrophobic environment lend further support that salivary histatins may act by altering membrane permeability and the ionic gradient in C. albicans. However, further experimental evidence is necessary to determine the mechanism of interaction of histatins with C. albicans. Although most of the peptide antibiotics of microbial origin are highly toxic, histatins, as salivary components, are nontoxic. The structural flexibility of histatins may permit them to maintain an unordered state in the aqueous environment of the oral cavity, but adopt a helical conformation in the hydrophobic environment of the candidal cell membrane. This structural flexibility, as observed from the CD studies, may be the reason for the remarkable lack of toxicity of salivary histatins.

In summary, the present results on the studies of histatin 5 and its fragments show that the sequence at the C-terminal (9-24) of histatin 5, a peptide chain length of 14 residues and helical conformation are the major structural requirements for eliciting appreciable candidacidal activity. The high biological activity of the C-terminal fragments C14 and C16 suggests that histidines at positions 15, 18, 19, and 21 and lysines at 11 and 13 and arginine at 12 in histatin 5 are functionally important for candidacidal activity. There exists an excellent correlation between the tendency of the peptides to form helical conformations due to increased chain length and candidacidal activity. The present studies also suggest that histatins which favor helical conformations in a non-aqueous hydrophobic environment may interact with C. albicans by altering the permeability of the cell membrane and ionic gradient across cell membranes.

**REFERENCES**

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Oppenheim, F. G., Yang, Y. C., and Troxler, R. F. (1965) J. Dent. Res. 64, 239
Synthesis, CD, and Structure-Function Analyses of Salivary Histatin 5 and Fragments


Supplemental Material To:

Salivary Histatin 5 - Dependence on Sequence, Chain Length and Helical Conformation for Candidal Activity


**EXPERIMENTAL PROCEDURES**

**Selection of Peptides** - Among the free histidines whose primary sequences (Fig. 1, Table I) are known, histatin 5 has been chosen to be the most effective in killing homologous G. lamblia (Oppenheim et al., 1988). Hence, this sequence was chosen for investigation together with G. lamblia 18 (model fragment, G. lamblia residues 25-41, 74-106, and 122-136) as a representative of the G. lamblia microorganism. All peptide samples were purified and assayed on a Bio-Rad Microplate Spectroflow unit with an Applied Biosystems 480B

**Pepstatin Peptide Synthesis and Purification** - The peptides were synthesized by solid-phase techniques using phosphoramidite resin and N-nitrobenzylaminophenol (N-bz) resin acids. The side chain protecting groups were the following: N-benzyl (Bz), N-nitrobenzyl (N-bz), N-nitrobenzyl (N-bz), N-acetyl (Ac), N-hydroxybenzotriazole (HBT), and N-carboxybenzyl (Cbz). The coupling reactions were carried out with a 1:1:1:1 molar ratio of porous acid in peptide, and in dichloromethane using diisopropylcarbodiimide and the coupling solvent, the symmetrical o-thiolhexane coupling (Buckwaltz and Young, 1984). Purifying proteins were cellulose and 25% solids of dihydrochloric acid instead of dichloromethane. The purest histatin 5 was then used with appropriately hydrogenated toluene for 1 h at 0°C. After removing the hydrogenated toluene, the sample was washed with water and the peptides extracted with water containing 1% triethanolamine and triethanolamine solution by centrifugation. Peptides were purified by reverse-phase high-performance liquid chromatography.

The homogeneity and the sequence of the histatin peptide were verified by sequence analysis. In addition, the amino acid composition of Histatin 5 and its fragments were found to be consistent with their sequences. The synthetic histatin 5 showed a single band in a calcium phosphate system (Bours et al., 1987), which is similar to the native histatin 5 (Fig. 3). The purity of the histatin 5 (Fig. 8) was also checked by comparing its UV spectrum with that of the native histatin 5 from human parotid native (Fig. 8).

A mixture of natural histatin (1+2 mg) was obtained by separation from other human parotid saliva proteins (0.2 mg) by gel filtration on a column (2.3·250) of Sephadex G-25 using 50 mM ammonium formate, pH 4.0 (Oppenheim et al., 1988). These were dissolved in 10% acetic acid, and subjected to reverse-phase HPLC using sodium chloride (0.1%)/water (0.99%) gradient eluents eluted at 0.01 M sodium chloride at 380 nm. A 90 mm gradient was run as follows for equal volumes: 100% sodium chloride to 100% water (0.99%) and 30% acetic acid (0.1%) for 30 min. Fig. 1 shows the HPLC profile of the authentic histatin 5, which is consistent with the elution profile of the synthetic histatin 5. peaks, which correspond to natural histatin 5 and eluted separately and resolved as shown in Fig. 2. The HPLC retention time observed under identical conditions for natural histatin 5 is in good agreement with that of the synthetic histatin 5. The purity of the synthetic histatin 5 was 95% as determined by gel electrophoresis. The purity of the synthetic histatin 5 was also established by comparing its CD activity with that of the native histatin 5 (Fig. 2) and by comparing its CD activity at 222 and 208 nm with those of the salivary histatin 5 (Fig. 3).

**Organisms and Growth Conditions** - Candida albicans strain CBS 5066 was obtained from the American Type Culture Collection, Rockville, MD (ATCC 56416) and grown on a lactose-containing medium at 37°C. One colony of C. albicans from this plate was inoculated into 10 ml of yeast synthetic growth medium containing lactose (50 ml/ml). The suspension of yeast cells was used to inoculate 10 ml of yeast synthetic growth medium containing lactose (50 ml/ml). Cells were counted and used for the experiments. The suspension of yeast cells was grown overnight at 37°C, and activity of the test peptide was calculated in the ratio of colonies per test plate to the number of colonies on control (no peptide plates).
Synthesis, CD, and Structure-Function Analyses of Salivary Histatin 5 and Fragments

Fig. 8 HPLC elution profile of (a) mixture of histatines separated from human periodontal saliva on a Lichrosorb RP 18 column (5 μm, 250 mm) coupled to a guard column (5 μm, 50 mm) using acetonitrile and water (both containing 0.1% TFA) as the solvent system and a flow rate of 1.2 ml/min. The gradient used is indicated by dotted lines in the solvent program. Peptides were derivatized at 280 nm. HPLC traces of (b) purified native histatin 5 (250 μg) which correspond to peaks II and (c) purified synthetic histatin 5 (250 μg) under identical conditions. All samples were loaded onto an injector loop of 500 μl capacity.

Fig. 9 Reverse phase HPLC traces of purified fragments of histatin 5 using acetonitrile and water (both containing 0.1% TFA) as the solvent system. (a) Buffer A (pH 7.4, 1.5 ml/min) and Buffer B (pH 7.4, 1.5 ml/min) Gradients used in indicated by dotted lines in the solvent program. Peptides were derivatized at 260 nm. Samples (500 μg) were loaded onto an injector loop of 500 μl capacity.
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