Structure-Activity Determinants in Paneth Cell α-Defensins

LOSS-OF-FUNCTION IN MOUSE CRYPTDIN-4 BY CHARGE-REVERSAL AT ARGININE RESIDUE POSITIONS*

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Paneth cells secrete microbicidal enteric α-defensins into the small intestinal lumen, and cryptdin-4 (Crp4) is the most bactericidal of the mouse α-defensin peptides in vitro. Here, site-directed Arg to Asp mutations in Crp4 have been shown to attenuate or eliminate microbicidal activity against all of the bacterial species tested regardless of the Arg residue position. R31D/R32D charge-reversal mutagenesis at the C terminus and mutations at R16D/R18D, R16D/R24D, and R18D/R24D in the Crp4 polypeptide chain eliminated in vitro bactericidal activity, blocked peptide-membrane interactions, as well as Crp4-mediated membrane vesicle disruption. Lys for Arg charge-neutral substitutions in (R16K/R18K)-Crp4 did not alter the bactericidal activity relative to Crp4, showing that bactericidal activity appears not to require the guanidinium side chain of Arg at those two positions. Partial restoration of (R31D/R32D)-Crp4 bactericidal activity occurred when an electropositive Arg for Gly substitution was introduced at the peptide N terminus and the (G1R/R31D/R32D)-Crp4 peptide exhibited intermediate membrane binding capability. Also, the loss of peptide bactericidal activity in (G1D/R31D/R32D)-Crp4 and (R16D/R24D)-Crp4 mutants corresponded with diminished phospholipid vesicle disruptive activity. Fluoresphore leakage from anionic phospholipid vesicles induced by the charge-reversal variants was negligible relative to Crp4 and lower than that induced by pro-Crp4, the inactive Crp4 precursor. Thus, Arg residues function as determinants of Crp4 bactericidal activity by facilitating or enabling target cell membrane disruption. The role of the Arg residues, however, was surprisingly independent of their position in the polypeptide chain.

Paneth cells at the base of the crypts of Lieberkühn in the small intestine secrete apically oriented granules as components of innate immunity. The secretory granules are charged in response to cholinergic stimulation or exposure to bacteria or their antigens (1–3), and they contain several antimicrobial peptides and proteins (4) including lysozyme (5–7), secretory phospholipase A₂, angiotensin (8), and α-defensins (9–12).

The α-defensins are cationic, amphipathic 3–4-kDa peptides with a defining trisulfide arrangement and β-sheet polypeptide backbone that have a broad spectrum of antimicrobial activities (13–15). For example, mouse α-defensins, termed cryptdins (Crps),¹ account for ~70% of the bactericidal peptide activity in secretions elicited from Paneth cells (3) and Crp4 is the most potent of the known mouse α-defensin peptides (16, 17). Also, Crps became implicated as components of mouse innate enteric immunity in vivo when mice that lacked matrix metalloproteinase-7, the pro-Crp-activating enzyme, were shown to have impaired host defense against oral infections (18, 19). Remarkably, mice transgenic for human Paneth cell α-defensin HD5 expressed the minigene specifically in Paneth cells and they were immune to oral infection by high doses of Salmonella enterica serovar Typhimurium (S. typhimurium) (20). Accordingly, an understanding of structure-activity relationships in α-defensins will improve the understanding of mucosal innate immune mechanisms.

To investigate the role of α-defensin primary structure in innate immunity, amino acid substitutions that alter charge were introduced into the Crp4 peptide and tested for effects on microbicidal activity and on Crp4-membrane interactions. Regardless of the site of mutagenesis, charge-reversal substitutions at Arg positions altered Crp4 bactericidal activity profoundly and the loss of activity correlated directly with quantitative effects on peptide binding to phospholipid vesicles and with peptide-induced vesicular permeabilization.

**EXPERIMENTAL PROCEDURES**

Preparation of Recombinant Crp4 Peptide Variants—Recombinant Crp4 peptides were expressed in Escherichia coli as N-terminal His₆/-tagged fusion proteins from the EcoRI and SalI sites of the pET23a expression vector (Novagen, Inc., Madison, WI) as described previously (21, 22). The Crp4-coding CDNA sequences were amplified using forward primer [ER1-Met-C4-F], 5’-GCGCGATATTCATCGAGGGAAAG-GATGGTTTGTGTATGCTAGTGT-3’, paired with reverse primer [pMAL-CP4-R1], 5’-ATAATATGTCGACTCATGACGACAGACCGGTGT-ACAAAAAGT-3’.

¹ The abbreviations used are: Crp, cryptdin; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; %CR, percent colorimetric response; PIPES, 1,4-piperazinediethanesulfonic acid; CFU, colony forming units; PDA, polydiacetylene; LUV, large unilamellar phospholipid vesicles; ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid.

This paper is available on line at http://www.jbc.org/
Peptide Interactions with Phospholipid/Polydiacetylene (PDA) Mixed Vesicles—Crp4 and three mutant peptides with varied Arg charge reversals were investigated for their relative membrane perturbation activities. Colorimetric phosphor/PDA vesicles were prepared using dimyristoylphosphatidylcholine (Sigma) as described previously (22, 23). Pro-Crp4 and phospholipid to vesicles were prepared by dissolving the phospholipids and 10,12-tricosadiynoic acid monomer (GFS Chemicals, Powell, OH) together in chloroform/ethanol (1:1), drying in vacuo to constant weight, suspending in H2O, probe-sonicating for 3 min at 70 °C, and incubating overnight. PDA was polymerized by irradiation at 254 nm for 10–20 s, producing suspensions with an intense blue appearance. Peptides (0.2–20 μM) were added to 60 μl of vesicle solutions (0.5 mM total lipid) in 25 mM Tris-HCl (pH 8) and diluted to 1 ml, and spectra were acquired at 28 °C between 400 and 700 nm on a Jasco V-550 spectrophotometer (Jasco Corp., Tokyo, Japan) using a 1-cm optical path cell. Blue-to-red color transitions within the vesicle solutions, defined as the percent colorimetric response (ΔCR), were calculated as described previously (22, 23).

Fluorescence-based Vesicle Leakage Assays of Peptide-Membrane Interactions—Crp4, (R13D/R32D)-Crp4, (G1D/R13D/R32D)-Crp4, (R16D/R24D)-Crp4, and pro-Crp4 were tested for their relative abilities to induce leakage from large unilamellar phospholipid vesicles (LUV) of defined composition. LUV of palmitoyl-oleoyl-phosphatidylglycerol (Avanti Polar Lipids, Birmingham, AL) were loaded with a fluoresphore/quencher system (27, 28). Aqueous lipid solutions consisting of 17 mM 8-amino-1-nonanethane-1,3,6-trisulfonic acid (ANTS, Molecular Probes, Eugene, OR), 60.5 mM DPX (p-xylene-bis-gyridinium bromide, Molecular Probes, Molecular Probes), 10 mM HEPES, 31 mM NaCl, and 19.5 mM NaOH (260 μs/mliter, pH 7.4) were vortexed, frozen, and thawed for five cycles and then polymerized through 100-nm pore size polycarbonate filters. Vesicles were separated from unencapsulated ANTS/DPX by gel-permeation chromatography from 130 mM NaCl, 10 mM HEPES, and 4.5 mM NaOH (260 μs/mliter, pH 7.4) as column eluant. Vesicular suspensions diluted with eluant buffer to ~74 μl of total lipid were incubated with peptides at ambient temperature. Time-dependent fluorescence produced by ANTS release was monitored at 520 nm (excitation at 353 nm) as described previously (24, 28). The kinetics of vesicular leakage was a function of peptide concentration, and equilibrium was attained ~4 h. Thus, 4-h values were expressed relative to fluorescence obtained by vesicular solubilization with Triton X-100.

RESULTS

Bacterial Activities of Recombinant Crp4 Variants—Recombinant peptides (Fig. 1A) were prepared using the pET-28 vector system (21). All of the peptides were purified to homogeneity by reverse-phase high performance liquid chromatography after chemical cleavage with CNBr, migrating as single entities and as expected relative to native Crp4 and pro-Crp4 molecules (21, 24). Purity was verified by analytical reverse-phase high performance liquid chromatography (data not shown) and acid-urea-PAGE analyses (Fig. 1B). The molecular masses of individual recombinant peptides were determined by MALDI-TOF mass spectrometry, and they matched the respective theoretical values. Thus, the purified recombinant peptides were homogeneous (Fig. 1B) and their biochemical features were consistent with the modifications introduced to the natural Crp4 molecule (29).

As a first step toward investigating structure-activity relationships in Crp4, the in vitro microbial activities of Crp4, pro-Crp4, and selected N-terminal Crp4 variants were measured against a panel of bacterial test species (data not shown) (24). The overall bacterial activities of the N-terminal variants differed only slightly over a range of 0.6–10 μg/ml of peptide as observed previously (24). In contrast to the bacterial activities of these mature Crp4 peptide variants, pro-Crp4 lacks microbial activity because matrix metalloproteinase-
**Fig. 1. Recombinant Crp4 and Crp4 variants prepared by site-directed mutagenesis.** Panel A, the primary structures of the recombinant Crp4 peptides prepared and investigated in these studies are aligned with the exception of pro-Crp4, which is not shown. Numerals below the Crp4 sequence refer to residue positions at the beginning of N-terminal sequences detected in the digests numbered with the native Crp4 N-terminal Gly as residue position 1. Altered residues in mutant Crp4 peptides are shown in **underlined boldface** representing deleted residues. The conserved a-defensin intramolecular disulfide bond arrangement is identified by the connecting bars above the Crp4 sequence. Panel B, 2-μg samples of purified recombinant Crp4, Crp4 variants, and pro-Crp4 were resolved by acid-urea-PAGE and stained with Coomassie Blue. Lane 1, pro-Crp4 (arrowhead); lane 2, Crp4 (lower arrow); lane 3, (G1D)-Crp4; lane 4, (G1V)-Crp4; lane 5, (G1R)-Crp4; lane 6, (G1R/G31/G32)-Crp4; lane 7, (R31G/R32G)-Crp4; lane 8, (R31D/R32D)-Crp4; lane 9, (ΔR31/R32)-Crp4; lane 10, (G1R/R31D/R32D)-Crp4; lane 11, (R31D/R32D)-Crp4; lane 12, (Arg-33/Arg-34)-Crp4; lane 13, (Arg-33/Arg-34)-Crp4; lane 14, (R16D/R18D/R18D)-Crp4; lane 15, (R16D/R24D)-Crp4; lane 16, (R16V/R18D/R18D)-Crp4; lane 17, (R16D/R24D)-Crp4; lane 18, (R16D/R24D)-Crp4; lane 19, (R16D/R24D)-Crp4; lane 20, (R16D/R24D)-Crp4; lane 21, (R16D/R24D)-Crp4; lane 22, (R16D/R24D)-Crp4; lane 23, (G1D)-Crp4; lane 24, (G1V)-Crp4; lane 25, (G1R)-Crp4; lane 26, (G1R/G31/G32)-Crp4; lane 27, (R31G/R32G)-Crp4; lane 28, (R31D/R32D)-Crp4; lane 29, (ΔR31/R32)-Crp4; lane 30, (Arg-33/Arg-34)-Crp4.

7-mediated proteolysis is required for Crp4 activation (18, 19, 21). Although the (G1D)-Crp4 peptide was consistently less active at ≤5 μg/ml of peptide (data not shown), modifications at the Crp4 N terminus had very modest effects on bactericidal activity (24).

**Modifications at the Crp4 C terminus—**Because the Cys-1 to Cys-6 disulfide bond common to α-defensins places the Crp4 N and C termini in proximity (13), we prepared a series of Crp4 peptide variants with charge-modified C termini including (R31D/R32D)-Crp4, (R31G/R32G)-Crp4, (R31V/R32V)-Crp4, and (ΔR31/R32)-Crp4 (Fig. 1). Bactericidal activity assays of these variants showed that deleting the two C-terminal Arg residues or converting them to Gly or Val altered peptide activity variably depending on the bacterial target species (Fig. 2, compare B with D). In contrast, however, the R31D/R32D charge-reversal mutation eliminated bactericidal activity, even against the S. typhimurium PhoP strain (data not shown), which is very sensitive to membrane-active cationic peptides (Fig. 2) (30, 31). As expected, wild-type S. typhimurium and the PhoP-constitutive PhoP+ CS022 strain were less sensitive to all of the peptides (data not shown).

Additional (R31D/R32D)-Crp4 variants with modified N termini were prepared and analyzed including an Arg1 to Gly1 substitution ([G1D/R31D/R32D]-Crp4) and an Asp7 for Gly7 variant ([G1D/R31D/R32D]-Crp4). Under the conditions of the assays, no bactericidal activity was detected with the (G1D/R31D/R32D)-Crp4 peptide but the (G1R/R31D/R32D)-Crp4 activity improved with the added electropositive side chain at the N terminus (Fig. 3). These findings showed that modifications of combined N- and C-terminal charge can modulate Crp4 bactericidal activity.

To investigate the effect of added C-terminal Arg residues on Crp4 bactericidal activity, additional modifications were introduced at the Crp4 C terminus. C57BL6 mice express a Crp4 variant (Crp4(B6b)) that has four Arg residues at its C terminus (21), but because Crp4 and Crp4(B6b) differ at several other positions, the specific role of C-terminal charge in these peptides cannot be compared directly. Accordingly, the natural Crp4 C terminus was extended by two Arg residues to place the Crp4(B6b) C terminus in the context of the Crp4 primary structure. The peptide, (Arg-33/Arg-34)-Crp4, had greater in vitro bactericidal peptide activity than Crp4 against E. coli and wild-type and PhoP+ S. typhimurium strains (Fig. 4), suggesting that C-terminal charge could be a determinant of bactericidal activity. However, the Crp4(B6b) molecule lacks the Pro-30 residue present in Crp4 between Cys-29 and Arg-31. Therefore, the effect of the Pro-30 residue was tested by assaying the bactericidal activity of (ΔPro-30/Arg-33/Arg-34)-Crp4, a variant of (Arg-33/Arg-34)-Crp4 from which Pro-30 was deleted (Fig. 1). (ΔPro-30/Arg-33/Arg-34)-Crp4 was less active than Crp4 and (Arg-33/Arg-34)-Crp4 against E. coli and S. typhimurium PhoP+ (Fig. 4), and the results in Fig. 4 also are representative of the relative bactericidal activities of these peptides against V. cholerae, S. aureus, and L. monocytogenes.
Thus, addition of Arg residues at the C terminus per se is not sufficient to improve peptide activity in the absence of proline at residue position 30. Because the R31D/R32D C-terminal charge reversal eliminated Crp4 bactericidal activity, the effects of charge alterations at other residue positions in the Crp4 polypeptide chain were studied to test whether the effects were specific to C-terminal modification.

![Fig. 2](image-url) Bactericidal activity of recombinant Crp4 and C-terminally modified Crp4 variants. Exponentially growing V. cholera (A), E. coli ML 35 (B), L. monocytogenes (C), and S. aureus (D) were exposed to the peptide concentrations shown: 50 μl of 10 mM PIPES (pH 7.4), 1% trypticase soy broth for 1 h at 37 °C (see “Experimental Procedures”). Following exposure, bacteria were plated on semi-solid media and incubated for 16 h at 37 °C. Surviving bacteria were quantitated as CFU/ml for each peptide concentration. Colony counts below $1 \times 10^3$ CFU/ml indicate that no colonies were detected. Symbols: Crp4 (-G-); (R31D/R32D)-Crp4 (-E-); (R31G/R32G)-Crp4 (-/c141-); (Arg-31/Arg-32)-Crp4 (-/f-); and (R31V/R32V)-Crp4 (-). Against all of the species tested, the (R31D/R32D)-Crp4 charge-reversal mutation attenuated bactericidal activity to the greatest extent.

![Fig. 3](image-url) Introduction of N-terminal charge alters bactericidal activity of C-terminal Crp4 mutants. Exponentially growing V. cholera (A), E. coli ML 35 (B), L. monocytogenes (C), and S. aureus (D) were exposed to the peptides, and surviving bacterial cells were quantitated as CFU/ml (Fig. 2). Symbols: Crp4 (-G-); (R31D/R32D)-Crp4 (-E-); (G1D/R31D/R32D)-Crp4 (-/c141-); and (G1R/R31D/R32D)-Crp4 (-/f-). (G1R/R31D/R32D)-Crp4 has bactericidal activity intermediate between that of Crp4 and (R31D/R32D)-Crp4, and the addition of the (G1D) mutation to (R31D/R32D)-Crp4 abrogates activity.
Charge Reversal at Arg Positions Result in Loss-of-function—
Crp4 mutants with double Asp for Arg substitutions were introduced at three additional Arg pairs, purified, and assayed for bactericidal peptide activity (see “Experimental Procedures”). As was observed for (R31D/R32D)-Crp4, the R16D/R18D, R16D/R24D, and R18D/R24D variants of Crp4 all lacked bactericidal activity under the conditions of these in vitro assays (Fig. 5). Thus, the loss of activity by charge reversal is not a specific effect at the C terminus, because all of the peptides with double charge-reversal mutations were inactive. Perhaps, Asp for Arg substitutions eliminate bactericidal activity by reducing the overall electropositivity of the Crp4 peptide. Nevertheless, it is unlikely that Arg residues at specific positions are required for Crp4 bactericidal activity because all of the mutations introduced resulted in equivalent loss-of-function independent of the position of the replacements.

To determine whether a specific requirement exists for the guanidinium group of the Arg side chain in Crp4 or whether charge-neutral substitutions at Arg positions would result in peptides with equivalent bactericidal activity, we prepared (R16K/R18K)-Crp4 (Fig. 1A) and evaluated its antimicrobial activity. Because all of the Crp4 charge-reversal mutants were attenuated similarly including (R16D/R18D)-Crp4, the (R16K/R18K)-Crp4 internal charge-neutral mutant was taken to be representative of such peptide alterations. As stated earlier, the Lys for Arg substituted peptide was purified to homogeneity as in Fig. 1B (data not shown) prior to comparing its bactericidal peptide activity relative to Crp4. The bactericidal peptide activities of Crp4 and (R16K/R18K)-Crp4 were not distinguishable against E. coli or L. monocytogenes (Fig. 6) or against other bacterial species (data not shown). Thus, with regard to bactericidal peptide activity, Lys could replace Arg in Crp4 without effect.

**Fig. 4.** C-terminal cationicity is insufficient to augment Crp4 bactericidal activity. Exponentially growing E. coli ML 35 (A) and S. typhimurium (PhoP+) (B) were exposed to the peptides, and surviving bacterial cells were quantitated as CFU/ml (Fig. 2). Symbols: Crp4 (○); (Arg-33/Arg-34)-Crp4 (△); and (ΔPro-30/Arg-33/Arg-34)-Crp4 (□). C-terminal addition of Arg residues does not augment bactericidal activity in the absence of Pro-30.

**Fig. 5.** Equivalent loss-of-function by charge reversal at multiple Arg residue positions in Crp4. Exponentially growing V. cholerae (A), E. coli ML 35 (B), L. monocytogenes (C), and S. aureus (D) were exposed to the peptides, and surviving bacterial cells were quantitated as CFU/ml (Fig. 2). Symbols: Crp4 (●); (R16D/R18D)-Crp4 (△); (R16D/R24D)-Crp4 (○); (R16D/R24D)-Crp4 (△); (R16D/R24D)-Crp4 (○); (R16D/R24D)-Crp4 (△); and (R16D/R24D)-Crp4 (□). All pairs of Asp for Arg charge reversals eliminate bactericidal activity of the parent Crp4 peptide.

Charge-reversal Mutations Block Crp4-Membrane Interactions—To investigate the mechanisms by which charge-reversal mutagenesis of Crp4 eliminates peptide activity, we compared interactions of Crp4 and charge-reversal Crp4 mutants...
with membranes using colorimetric binding assays based upon lipid/PDA-mixed vesicles (22) and leakage assays from large unilamellar vesicles (24–28).

Crp4 loss-of-function by Arg to Asp mutagenesis correlated with the inability of mutant peptides to interact with membranes at the vesicular lipid/water interface and to perturb the hydrophobic membrane core. In the dimyristoylphosphatidylcholine/PDA-mixed vesicle system, higher %CR values corresponded to interfacial lipid binding because peptides that localized at the lipid bilayer surface induced greater perturbation in the head-group region of the lipid/polymer assembly than peptides that penetrate deeper into the hydrophobic core (32, 33). Crp4 induced blue-red chromatic transitions, reaching a plateau of ~35%CR upon interaction with the phospholipid/PDA vesicles. The %CR value was dependent on peptide concentration (Fig. 7), and previous work has determined that the colorimetric response indicates an interface localization of Crp4 (22, 32). In contrast and without exception, only base-line %CR values were induced by inactive Crp4 mutants with Asp for Arg substitutions (Fig. 7). Consistent with its intermediate bactericidal activity (Fig. 4), (G1R/R31D/R32D)-Crp4 induced somewhat higher intermediate %CR values between those of Crp4 and the Crp4 variants (Fig. 7). Thus, the loss of bactericidal activity by Arg charge reversals was consistent with defective Crp4 interaction with the vesicular lipid/water interface and an inability to perturb the membrane core of the leaflet.

The membrane-disruptive activities of Crp4 and inactive Crp4 variants were analyzed by comparing induced leakage of the low molecular weight fluorophore, ANTS, from LUV (see “Experimental Procedures”). Crp4 induces leakage from LUV via a "graded leakage" mechanism (24, 28), and pro-Crp4, consistent with its lack of bactericidal activity (21, 22), induces markedly less leakage than Crp4. For both peptides, leakage is dependent on LUV phospholipid composition (Fig. 8) (28). When exposed to (R31D/R32D)-Crp4, (G1D/R31D/R32D)-Crp4, and (R16D/R24D)-Crp4, ANTS-loaded LUV prepared with the anionic lipid palmitoyl-oleyl-phosphatidylglycerol exhibited leakage that was ~5% of that induced by Crp4, much less than leakage induced by pro-Crp4 (Fig. 8). Thus, regardless of the position of Arg charge reversals in the polypeptide chain, the loss-of-function was the same and corresponded directly to a loss of peptide binding to membranes and an inability to disrupt phospholipid vesicles in vitro.

**DISCUSSION**

In this report, site-directed Arg to Asp mutations in Crp4, a mouse Paneth cell α-defensin, were shown to attenuate or eliminate microbicidal activity against all of the bacterial target cells examined. Whether charge reversals occurred at the C terminus, at R16D/R18D, R16D/R24D, or at R18D/R24D, mutagenesis eliminated Crp4 in vitro bactericidal activity, blocked peptide-membrane interactions, and eliminated Crp4-mediated membrane vesicle disruption. Because Lys for Arg charge-neutral substitutions in the (R16K/R18K)-Crp4 peptide did not modify Crp4 bactericidal peptide activity, it appears that it is the charge rather than a specific requirement for Arg per se that determines bactericidal activity in Crp4. Fluorophore leakage from anionic phospholipid vesicles induced by the charge-reversal variants was negligible relative to Crp4 and lower than that induced by pro-Crp4, the inactive Crp4 precursor. Thus, Arg residues function as determinants of bactericidal activity in native Crp4 by facilitating or enabling target cell membrane disruption but the role of the Arg residues appears to be independent of their position in the polypeptide chain.

The findings reported here support the conclusion that Arg residues in the Crp4 polypeptide chain facilitate peptide-membrane binding and interactions that lead to or are coincident with disruption of phospholipid bilayers (Figs. 7 and 8). Despite the insights provided by α-defensin crystal and solution structures (14, 34–36), structure-activity relationships in the α-defensin peptide family remain obscure. The possibility that Asp for Arg-substituted Crp4 molecules expressed in E. coli may...
not be folded properly or form a correct disulfide array is a potential concern. However, preliminary one- and two-dimen-
sional TOCSY NMR determinations indicate that the mutant peptides have proper disulfide connectivities.2 Also, when nat-
ural α-defensins have been available as standards, recombi-
nant mouse α-defensins and their precursors that we have prepared co-elute from reverse-phase high performance liquid chromatography. The recombinant peptides also co-migrate in reverse-phase high performance liquid chromatography. The recombinant peptides also co-migrate in

dimensions. Neutrophil α-defensins permeabilize the outer and inner membranes of E. coli sequentially, inducing the forma-
tion of ion channels in lipid bilayers (48), and both of these peptide-elicited effects are influenced by membrane energetics (49). The crystal structure of human neutrophil peptide 2 is a noncovalent dimer (14), and human neutrophil peptide 2 dimers form stable 20-Å multimeric pores in LUV after inser-
tion into model membranes (40). In contrast, rabbit neutrophil peptide 1 is a monomer in solution (35) and neutrophil peptide 1 permeabilizes the membrane by creating large short-lived defects in model phospholipid bilayers (50). Although the struc-
ture of Crp4 is not yet reported, the peptide, which has the greatest in vitro bactericidal activity among the mouse α-de-
fen sin s, exhibits strong interfacial binding to model mem-
bbranes (22) and induces graded fluorophore leakage from LUV (24, 28). Thus, Crp4 resembles the rabbit neutrophil peptide 1-type α-defensin mechanism of bactericidal action and site-
directed Arg to Asp mutagenesis targets Crp4 residues that facilitate or enable that membrane-disruptive mechanism.

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Structure-Activity Determinants in Paneth Cell α-Defensins: LOSS-OF-FUNCTION IN MOUSE CRYPTDIN-4 BY CHARGE-REVERSAL AT ARGININE RESIDUE POSITIONS

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