Targeting Host Cell Furin Proprotein Convertases as a Therapeutic Strategy against Bacterial Toxins and Viral Pathogens*§

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Pathogens or their toxins, including influenza virus, Pseudomonas, and anthrax toxins, require processing by host proprotein convertases (PCs) to enter host cells and to cause disease. Conversely, inhibiting PCs is likely to protect host cells from multiple furin-dependent, but otherwise unrelated, pathogens. To determine if this concept is correct, we designed specific nanomolar inhibitors of PCs modeled from the extended cleavage motif TPQERRKKKR (R/K/X)GL of the avian influenza H5N1 hemagglutinin. We then confirmed the efficacy of the inhibitory peptides in vitro against the fluorescent peptide, anthrax protective antigen (PA83), and influenza hemagglutinin substrates and also in mice in vivo against two unrelated toxins, anthrax and Pseudomonas exotoxins. Peptides with Phe/Tyr at P1′ were more selective for furin. Peptides with P1′ Thr were potent against multiple PCs. Our strategy of basing the peptide sequence on a furin cleavage motif known for an avian flu virus shows the power of starting inhibitor design with a known substrate. Our results confirm that inhibiting furin-like PCs protects the host from the distinct furin-dependent infections and lay a foundation for novel, host cell-focused therapies against acute diseases.

Furin and related PCs are specialized serine endoproteases that cleave the multifunctional motifs RX(R/K/X)R and transform proproteins into biologically active proteins and peptides (1). Structurally and functionally, furin resembles its evolutionary precursor: the prohormone-processing kexin of yeast Saccharomyces cerevisiae. Furin is currently the most studied enzyme of the PC family. Seven PCs (furin, PC2, PC1/3, PC4, PACE4, PC5/6, and PC7) have been identified in humans (2). Furin is expressed in all examined tissues and cell lines and is mainly localized in the trans-Golgi network. Some proportion of the furin molecules cycles between the trans-Golgi and the cell surface. Because of the overlapping substrate preferences and cell/tissue expression, there is redundancy in the PC functionality, albeit certain distinct functions of the individual PCs have also been demonstrated. Furin knockout, however, is lethal in mice (3).

In addition to normal cell functions, PCs, including furin, are implicated in many pathogenic states, because they process to maturity membrane fusion proteins and pro-toxins of a variety of both bacteria and viruses, including anthrax and botulinum toxins and influenza A H5N1 (bird flu), flaviviruses, and Marburg and Ebola viruses (1). After processing by furin and the subsequent endocytic internalization in the complex with the respective cell surface receptor followed by acidification of the endosomal compartment, the processed, partially denatured, infectious proteins expose their membrane-penetrating peptide region and escape into the cytoplasm (4). The intact toxins and viral proteins are incapable of accomplishing these processes. Evidence suggests that the inhibition of cellular furin prevents aggressive disease (2, 5). These results lead to the logical suggestion that furin is a promising drug target in infectious diseases; an experimental confirmation, however, has been limited because research efforts have been focused primarily on anthrax (5–7). Because no natural protein inhibitors of furin are known, d-Arg-based peptides, α1-antitrypsin Portland, and the synthetic inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone (DEC-RVKR-CMK) are used in vitro and in cell-based tests (1, 2). Arg-based peptides such as hexa- and nona-d-Arg (8) have either low or no therapeutic potential because of their intrinsic ability to cross-react with multiple, pathogen and host, proteinase and non-proteinase targets, which are unrelated to furin (6, 9–11).

Here, we designed nanomolar peptide inhibitors modeled from the extended furin cleavage sequence of avian influenza A

* The abbreviations used are: PC, proprotein convertase; Cipro, ciprofloxacin; DEC-RVKR-CMK, decanoyl-Arg-Val-Lys-Arg-chloromethylketone; HA, hemagglutinin; LF, lethal factor; PA, protective antigen; PEx, Pseudomonas exotoxin A; Pyr-RTKR-AMC, pyrroglutamic acid-Arg-Thr-Lys-Arg-methylcoumaryl-7-amide; SiNPs, silica nanoparticles; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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H5N1 (12–14). We then proceeded to demonstrate the efficacy of the inhibitory peptides in assays in vitro and in cell-based and animal tests. Our results suggest that furin antagonists can provide host protection against multiple furin-dependent, but otherwise unrelated pathogens.

MATERIALS AND METHODS

Reagents—Reagents were purchased from Sigma unless indicated otherwise. A TMB/M substrate and GM6001 were from Chemicon. The inhibitor DECVKRM–CMK was obtained from Bachem. The protease inhibitor mixture set III and the fluorescence peptide substrate pyrogulatamic acid-Arg-Thr-Lys-Arg-Ala at the N terminus. Anthrax protective antigen-83 (PA83) and lethal factor (LF) were then added to the final concentrations of 500 ng/ml and 25 ng/ml, respectively. After incubation for an additional 1 h, cell viability was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. Cells were incubated with 0.5 mg/ml MTT in Dulbecco’s modified Eagle’s medium for 45 min at 37 °C; the medium was aspirated, and the blue pigment produced by the viable cells was solubilized with 0.5% SDS/25 mM NaCl in 90% isopropl alcohol. The concentration of oxidized MTT in the samples was measured at 570 nm. Each datum point represents the results of at least three independent experiments performed in duplicate. The percentage of viable cells was calculated by using the following equation: (A570 of cells treated with LF, PA83, and inhibitor) − (A570 of cells treated with LF and PA83) − (A570 of cells treated with LF alone) − (A570 of cells treated with LF and PA83).

Animal Experiments with Anthrax Spores and Pseudomonas Toxin—To protect the peptide from proteolysis in vivo, the TPRARRKKRT peptide sequence was amidated at the C terminus and had β-Ala at the N terminus. Anthrax protective antigen-83 (PA83) and lethal factor (LF) were then added to the final concentrations of 500 ng/ml and 25 ng/ml, respectively. After incubation for an additional 1 h, cell viability was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. Cells were incubated with 0.5 mg/ml MTT in Dulbecco’s modified Eagle’s medium for 45 min at 37 °C; the medium was aspirated, and the blue pigment produced by the viable cells was solubilized with 0.5% SDS/25 mM NaCl in 90% isopropl alcohol. The concentration of oxidized MTT in the samples was measured at 570 nm. Each datum point represents the results of at least three independent experiments performed in duplicate. The percentage of viable cells was calculated by using the following equation: (A570 of cells treated with LF, PA83, and inhibitor) − (A570 of cells treated with LF and PA83) − (A570 of cells treated with LF alone) − (A570 of cells treated with LF and PA83). The TPRARRKKRT peptide alone when incubated with cells in concentrations up to 0.5 mM had no effect on cell viability.

Binding and Processing of PA83 by Cultured Cells—Glioma U251 cells (3 × 10⁶) were incubated for 3 h at 37 °C in serum-free Dulbecco’s modified Eagle’s medium supplemented with biotin-labeled PA83 (1 μg/ml). Where indicated, DEC-RVKR-CMK (20 μM) and the inhibitory peptides (2–20 μM) were added to the cells. After incubation, cells were washed and lysed in an radioimmunoprecipitation assay buffer (20 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, 1% IGEPAL, pH 7.4) containing a protease inhibitor mixture set III, 1 mM phenylmethylsulfonyl fluoride, and 10 mM EDTA. To measure cell-associated PA83 and PA63, the samples were analyzed by Western blotting with ExtrAvidin conjugated with horseradish peroxidase and a TMB/M substrate.

In Vitro Cleavage of PA83, HA, and PEx—PA83, HA, and Pseudomonas exotoxin A (PEx) were each labeled with EZ-Link sulfo-NHS-Long Chain(LC)-biotin from Pierce. Anthrax PA83 and Pseudomonas exotoxin A were purchased from List Biological Laboratories. Recombinant human furin, PC5/6, PC4, and PC7, and rat PACE4 were prepared in the S2 Drosophila expression system (Invitrogen) and purified to homogeneity as described previously (15).

Expression and Purification of Avian Influenza A H5N1 HA—The ectodomain of HA was cloned into the baculovirus pAcGP67A transfer vector (BD Biosciences) to allow for secretion of the recombinant protein. To facilitate the yield of the HA precursor, the C-terminal region of the contract contained the bacteriophage T4 fibritin “foldon” trimerizing sequence, a HA precursor, the C-terminal region of the contract contained the bacteriophage T4 fibritin “foldon” trimerizing sequence, a

Toxin—To protect the peptide from proteolysis in vivo, the TPRARRKKRT peptide sequence was amidated at the C terminus and had β-Ala at the N terminus. Purification of anthrax spores and the inhalation model of anthrax using A/J mice was described previously (17, 18). A/J mice (8 mice/group) received B. anthracis Sterne spores (4 × 10⁵) (animal in 20 μl of phosphate-buffered saline). On the day following infection, mice received the TPRARRKKRT peptide (12.5 mg/kg intraperitoneal) in phosphate-buffered saline and then continued to receive injections once daily for the remainder of the experiment. Control mice received an equal volume of phosphate-buffered saline. Mice treated with ciprofloxacin (Cipro) received 25 mg/kg subcutaneous treatments daily beginning on the fourth day following infection.

C57/BL6 mice (5 mice/group) received one intramuscular injection of PEx (500 ng/animal, 2 × LD₅₀) (19). Mice received one injection of the TPRARRKKRT peptide (12.5 mg/kg intraperitoneal) either 24 h prior to toxin injection or simulta-
neously with toxin injection. An additional group of mice, after receiving one injection of the peptide 24 h prior to toxin injection, continued to receive daily injections of the peptide for the remainder of the experiment.

**Peptides Synthesis**—A 96-well format centrifugal synthesizer and purification and characterization of the peptides were described earlier (20–22). Peptide synthesis was performed in wells of a 96-well flat bottom polypropylene microtitre plate (Evergreen Scientific). The peptides were amidated at the C terminus. In addition to the C-end amidation, peptides used for their attachment to silica nanoparticles (SiNPs) exhibited hydroxylaminoacetic acid at the N terminus (prepared by attachment of t-butoxycarbonyl-NHOCH₂-COOH at the last step of the synthesis). The purity of the peptides was confirmed by use of reversed-phase high-performance liquid chromatography and by mass spectrometry.

The peptide for the cell-based assays and in vivo studies was synthesized manually in a plastic syringe equipped with a frit (CSPS Pharmaceuticals) using Rink resin (1 g, 0.45 mmol/g, Novabiochem). Diisopropylcarbodiimide was used for coupling (2 × 1 h) and 20% 4-methylpipеридine (20, 23) for Fmoc (N-(9-fluorenyl)methoxycarbonyl) group deprotection. Final deprotection and cleavage from the resin was performed by using Mixture K (82.5% TFA, 5% phenol, 5% H₂O, 5% thioanisole, 2.5% ethanethiol) (24). The peptide sample was precipitated and washed (5×) in ether, dissolved in 0.1 M HCl, and lyophilized. The peptide was then dissolved in 10 ml of 0.1 M HCl and purified on a Sephadex LH-20 column equilibrated in 0.1 M HCl. Fractions containing the peptide were pooled and lyophilized. High-performance liquid chromatography (μBondapak C18, Waters, 10-μm particles, 125-Å pore size, 3.9 × 150 mm, gradient 0.05% trifluoroacetic acid in H₂O to 40% acetonitrile, 0.05% trifluoroacetic acid in 15 min, flow rate 1.5 ml/min, detection by UV at 217 nm) of the peptide determined the purity of the material to exceed 95%. Mass spectrometry analysis of the synthesized peptide confirmed the identity of the product (calculated molecular weight, 1495.81; found M + H, 1497).

**Protease Assays with Fluorescent Peptides**—The assay for PC activity was performed using a Pyr-RTKR-AMC substrate (24 μM). The concentrations of the catalytically active protein-ex and/or PCs were determined by log [I]t plots with sigmoidal dose-response curves, and the inhibition constant (Kᵢ) was derived using the Cheng-Prusoff equation: 

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Kᵢ = IC₅₀/(1 + [S]/Kₘᵢ)
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where Vo is the steady-state velocity of substrate hydrolysis, [I], is the total inhibitor concentration, [S] is the substrate concentration, Kₘᵢ is the Michaelis-Menten constant, and Kᵢ(app) is the apparent inhibition constant.

**Preparation of SiNPs and Immobilization of Peptides**—A 24.8 ml mixture (volume ratio, 4:2:1:1) was converted to a nanoemulsion by stirring at room temperature for 1 h. Water (940 μl) and tetramethyl orthosilicate (100.5 μl) were added. The mixture was sonicated for 1 h to facilitate the diffusion of tetramethyl orthosilicate into the encapsulated water droplets in the nanoemulsion. 28% NH₃ in water (59 μl) was added to catalyze the hydrolysis of tetramethyl orthosilicate and condensation to form the SiNPs. The reaction mixture was stirred for 24 h, followed by the addition of tetramethyl orthosilicate (10.05 μl) and then, in 30 min, aminopropyl trimethoxysilane (11.8 μl). The mixture was stirred for an additional 24 h, and then the amino-SiNPs were precipitated by 25-ml acetonitrile washed with water and anhydrous ethanol. The presence of amino groups on SiNPs was confirmed by using fluorescamine in methanol followed by sonication of the sample for 5 min at room temperature and fluorescence analysis (λ₂₅ = 390 nm, λ₄₅ = 475 nm). An aliquot of SiNPs in ethanol was placed on the lacy carbon film covering a 300-mesh copper grid (Ted Pella), and ethanol was then allowed to evaporate. Transmission electron microscopy images showed the uniform, 15 ± 1 nm diameter, aminosilanized SiNPs. Because the density of the SiNPs is equal to pure silica (1.96 g/cm³), the molecular weight of SiNPs was calculated to be 2000 kDa. 4-Formylbenzoyl chloride/triethylamine (1:3 molar ratio) was allowed to react with amino-SiNPs in dimethyl formamide for 40 min at 0 °C and then at room temperature overnight. Aldehyde-SiNPs were separated by the addition of water to the sample and extensively washed in water. To accomplish the binding of the peptides to aldehyde-SiNPs, a suspension of aldehyde-SiNPs (~0.2 mg/0.1 ml) was co-incubated for 48 h in a shaker with 1 mM solution of the peptides (which exhibited a hydroxylamine group) in 1 mM citrate buffer, pH 5.1-Me₃SO mixture (1:1, v/v). Beads were then centrifuged and washed with water.

**RESULTS**

**Derivatization of the Furin Cleavage Sequence**—Furin and related PCs are directly implicated in many pathogenic states, because they process membrane fusion proteins and pro-toxins of a variety of pathogenic bacteria and viruses, including anthrax and the highly pathogenic H5N1 avian influenza. Our results show that both PA83 and the HA precursor (HA0) from the H5N1 influenza virus (13, 14) are sensitive to the processing by several individual PCs as opposed to furin alone (Fig. 1).
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Peptide sequence and the $K_i$ values of the peptides against furin. Peptides exhibited the free N terminus, whereas the C terminus was amidated. The assay for PC activity was performed using a Pyr-RTKR-AMC substrate. Mutant amino acid positions and the most efficient $K_i$ values are shown in brackets. E, o-Glu; K, o-Lys; L, $\beta$-Ala; A, e-aminoheXanoic acid; G, aminocyclopentanecarboxylic acid; 4, citrullin; G, Cys(Me); and E, D-Glu; K, Nle.

| Peptide          | $K_i$ (nM) | $K_i$ (nM) |
|------------------|------------|------------|
| TPRARRKKR        | 0.07       | 0.047      |
| TPRARRRKRA       | >0.1       | 0.15       |
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| TPRARRRKRA       | >0.1       | 0.15       |

PA83 was converted to PA63 by furin and also by PC4 and PC5/6 (1 activity unit each) with an ~90% efficiency, whereas PACE4 and PC7 (1 activity unit each) accomplished a PA83-to-PA63 conversion with an ~70 and 40% efficiency, respectively. Both furin and PC5/6 were efficient in cleaving HA, whereas PACE4 and PC4 and, especially PC7, were much less efficient.

The pathogenicity of H5N1 correlates with the extended furin cleavage motif, TPQERRRRKRG cleavage motif led us to a potent inhibitor of furin (TPQERRRRKRG, $K_i = 57\text{ nm}$). The sequences and the $K_i$ values of the peptides are shown in Fig. 2, Table 1 and in supplemental Table S1. The inhibitory potency of TPQERRRRKRG against furin and other purified PCs (PACE4, PC4, PC5/6, and PC7) was improved further by substitution of the P1’ C-terminal glycine by several other amino acid residues, including threonine (TPQRRRRKRT, $K_i = 23\text{ nm}$) (Fig. 2 and supplemental Table S2). Other PCs (PACE4, PC4, PC5/6, and PC7) were also inhibited but with less efficiency (Table 1). Overall, peptides with aromatic C-terminal residues (Phe or Tyr) were more selective for furin, whereas TPQRRRRKRT and TPQRRRRKRG were potent pan-inhibitors of PCs ($K_i = 150–300\text{ nm}$) (Table 1). Co-incubation of the peptides with furin followed by mass spectrometry analysis showed that the inhibitory peptides were resistant to furin proteolysis (not shown).

**Cell-based and in Vivo Activity Tests**—We next determined if the HA-derived peptides could inhibit intoxication by two unrelated pathogens, anthrax and *Pseudomonas* toxins. Host cleavage of anthrax PA83 by PCs is a prerequisite for the translocation of the toxic enzymes, LF and edema factor, into the host cell cytosol (4). It is well established that, following its binding with the cell receptor, furin cleavage of PA83 occurs directly at the cell membrane rather than in the intracellular milieu (25). In our tests, U251 cells, which in addition to furin, PC5/6, and PC7 express sufficient levels anthrax toxin receptor (26), were allowed to bind and process PA83. The amounts of cell-associated PA83 and PA63 were determined by Western blotting.

Thus, using a cell-based assay in U251 cells, we determined that at a 20 M concentration TPQRRRRKRG peptides with C-terminal Phe, Trp, Thr, and Tyr accomplished a near complete inhibition of PA83 processing by cellular PCs (Fig. 3A). Consistent with inactivation of cell surface PCs and subsequent PA83 processing, the TPQRRRRKRG peptide inhibited delivery of the PA63-LF complex into the cytosol and protected cells from LF-induced cytotoxicity (Fig. 3B) with an efficiency similar to that of GM6001 (16). Because of its inhibitory activity (27), GM6001, a hydroxamate inhibitor of the LF metalloproteinase (2–5 M), also rescued cells from LF intoxication and was used as a control. The peptide alone at concentrations ≤0.5
mM displayed no toxicity and had no effect on cell viability (not shown).

We next tested the peptide in a mouse model of inhalation anthrax. A/J mice (8 mice/group) received B. anthracis Sterne spores (4 x 10^7/animal). On the day following infection, mice received the TPRARRKKRT peptide (12.5 mg/kg intraperitoneal) and then continued to receive injections once daily for the remainder of the experiment. Mice treated with antibiotic Cipro received 25 mg/kg subcutaneous treatments daily beginning on the fourth day following infection. This post-exposure peptide plus Cipro regimen protected 90% of the infected mice from disease, compared with 40% using either the peptide or Cipro alone (Fig. 3C).

We carried out a similar set of experiments with an unrelated toxin, Pseudomonas exotoxin A, the processing of which occurs in the intracellular milieu. Consistent with the earlier data (28–30), PEx was resistant to PC cleavage at pH 7.5 but following unfolding at pH 5.5 PEx (66 kDa) was readily processed by furin, PC4, and PC5/6 to produce the 28-kDa N-terminal fragment and the toxic 37-kDa C-terminal fragment (Fig. 4A). As expected, furin proteolysis of PEx was inhibited by the TPRARRKKRT peptide in the cleavage reaction in vitro (data not shown). To demonstrate the efficacy of the peptide in vivo, C57/B6 mice (5 mice/group) received one intramuscular injection of PEx (500 ng/animal, 2 x LD_{50}) (19) and one injection of the TPRARRKKRT peptide (12.5 mg/kg intraperitoneally) either 24 h prior to toxin injection or simultaneously with toxin injection. Another group of mice, after receiving one injection of the peptide 24 h prior to toxin injection, continued to receive daily injections of the peptide for the remainder of the experiment. Daily injections of the peptide provided protection (60% survival) from the lethal action of PEx, demonstrating efficacy against a second, otherwise unrelated, furin-dependent pathogen (Fig. 4B).

**Immobilization on the Peptides on Silica Nanoparticles—**

Given that cell surface-associated PCs in bronchial epithelial cells are the first to encounter inhaled pathogens, we suggest the development of an inhalation drug that could be used for acute treatment or for prophylactic use in civilian or battlefield settings. We investigated peptide immobilization on SiNPs, which have been widely used for biosensing and catalytic applications (31, 32). When peptides with either the GGG or the GGGGGG and GAGAGA linkers were immobilized on 15 nm diameter 4-formylbenzoyl chloride-activated SiNPs with a density of ~100 peptide molecules/particle (Fig. 5), the inhibitory efficacy of the immobilized peptides against furin was similar on a molar basis to that of the soluble peptides. Immobilization without a linker reduced the inhibitory efficacy (Table 2). Similar to soluble peptides, the immobilized TPRARRRRKRT peptide inhibited PEx processing by furin (Fig. 6). SiNPs showed no cell toxicity, even at high concentrations (e.g. 50 nM SiNPs (3 x 10^{17} SiNP particles)/100,000 cells).

**DISCUSSION**

Furin is a multidomain protease of animal cells, the catalytic domain of which is similar in structure to bacterial subtilisin. Furin is enriched in the Golgi apparatus and also on cell surfaces where it functions to cleave a number of functionally important cellular proteins, including proteinases, integrins, signaling receptors, growth factors, hormones, and neuropeptides, into their respective mature forms. In addition to furin, six additional, albeit less characterized, furin-related PCs are known in humans. In addition to processing cellular precursor proteins, PCs are also utilized by a number of pathogens. Pathogens or their toxins, including viruses and bacterial toxins,
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exploit host PCs to become fully functional and to allow entry into host cells and to cause disease onset. The low pathogenicity viral subtypes have mutations in the cleavage site sequence and thus a reduced sensitivity to furin (12). Overall, proteolytic processing by furin is an important determinant in the pathogenicity of viruses and bacterial toxins.

To develop sharply focused and specific antagonists of pathogen entry, we developed nanomolar range peptide inhibitors of host cell furin and furin-related PCs. For these purposes, we modified the sequence of the extended furin cleavage sequence of avian influenza A H5N1 HA. HA is synthesized as a precursor molecule (HA0), which undergoes proteolytic processing into two subunits (HA1 and HA2), which are held together by disulfide bonds (33). Without proteolysis, the acid-triggered conformational change of HA that occurs in the endosomes and that exposes the fusion peptide cannot occur, and the virus is non-infectious.

The resulting competitive inhibitors TPRARRKXR with C-terminal Phe, Trp, Thr, and Tyr were highly potent not only against furin but also against related PCs, including PACE4, PC4, PC5/6, and PC7. These inhibitors were capable of efficiently inhibiting furin proteolysis of anthrax PA83 and avian influenza H5N1 HA in vitro. The peptide inhibitors protected cells from LF-induced cytotoxicity.

Most importantly, we then confirmed the efficacy of the inhibitory peptides in mice in vivo against two unrelated toxins, anthrax and Pseudomonas exotoxin. Because cell surface-associated PCs in bronchial epithelial cells are the first to encounter inhaled pathogens, including influenza A H5N1 (bird flu), we developed an inhaled, nanoparticle-immobilized drug to minimize potential side-effects and optimize delivery. The specific furin inhibitors we designed are superior relative to D-Arg-peptides, which have been shown to cross-react with multiple host and pathogen targets, including both furin and anthrax lethal factor (6).

In summary, we have shown that peptides based on the cleavage motif of avian influenza H5N1 HA are efficient inhibitors of host cell furin and related PCs and that these inhibitors inhibit manifestation of toxicity by PC-depend-

TABLE 2

| K<sub>i</sub> values of the soluble and immobilized peptides against furin |
|------------------|------------------|------------------|------------------|------------------|
| Peptide         | N-terminal linker, K<sub>i</sub> μM |
|                 | None  | GGG- | GGGGGG- | GAGAGA- |
| Peptide alone   | TPRARRRKRT  | 0.023 | 0.039 | 0.066 | 0.047 |
| TPRARRRKRF      | 0.038 | 0.067 | 0.045 | 0.056 |
| TPRARRRKRY      | 0.047 | 0.042 | 0.054 | 0.057 |
| TPRARRRKRW      | 0.034 | 0.067 | 0.088 | ND   |
| Peptide on nanobeads | TPRARRRKRT  | 4.9  | 1.99  | 2.32  | 2.57  |
|                   | TPRARRRKRF  | 3.85 | 4.28  | 9.09  | ND*   |

* ND, not determined.
ent, but otherwise unrelated, pathogens. Our results support and extend the earlier, albeit less conclusive, observations by other authors (6–9, 34). Because furin is likely essential for many cell functions in adults, we suggest that our results represent a proof-of-principle from which novel, short-term therapeutics and prophylactics of furin-dependent acute disease pathogens, including anthrax, bird flu, Marburg, Ebola, and flaviviral infections will emerge (35).

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FIGURE 6. SiNPs with the immobilized peptides inhibit furin. SiNPs with the immobilized TPRARRRRKRT peptide inhibit furin processing of PA83 (500 ng). A 500 nM concentration of SiNPs (molecular mass, 2000 kDa) was used in the reactions.

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