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Effects of Magnesium Ions on Recombinant Human Furin: Selective Activation of Hydrolysis of Substrates Derived from Virus Envelope Glycoproteins

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Abstract

We report here a detailed analysis of Mg^{2+} ion effects on furin hydrolysis of fluorescent resonance energy transfer (FRET) decapeptide substrates derived from canonical R-X-K/R-R furin cleavage motifs within certain viral envelope glycoproteins and eukaryotic proproteins. Selective activation of furin by Mg^{2+} ions was observed using virus-derived sequences. Cooperativity between furin subsites was also observed during $MgCl_2$ activation. Furin hydrolysis of the peptides Abz-SRRHKR↓FAGV-Q-EDDnp (from measles virus fusion protein F_0), and Abz-RERRRKRR↓GLFG-Q-EDDnp (from Asian avian influenza A, H5N1) was activated between 60- and 80-fold by $MgCl_2$. It appears that virus envelope glycoprotein mutations have been selected to increase their susceptibility to furin within cells, a location where Mg^{2+} is present in adequate concentrations for activation. Both the pH profile of furin and its intrinsic fluorescence were modified by Mg^{2+} ions, which bind to furin with a K_d of 1.1 mM.

Keywords

Protease; peptidase; magnesium; fluorescence

Introduction

Furin (EC 3.4.21.75, MEROPS clan SB, family S8) is a Ca^{2+} -dependent serine endoprotease that cleaves protein precursors with a specificity that follows the multiple basic motif R-X-K/R-R (1), corresponding to the substrate positions P_4 to P_1 (nomenclature of Schechter and Berger) (2). The S_1 and S_4 furin subsites exhibit a stringent specificity for R while the S_2 preference is for K or R (3,4,5). Furin is present in the constitutive secretory pathway but also recycles along the trans-Golgi network (TGN), the cell surface and the endosomes. Furin processes soluble and membrane-bound precursors of many endogenous and secreted

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proteins, and is also involved in the processing of virus envelope glycoproteins, in the activation of several bacterial toxins, and in pathologies such as cancer and neurodegenerative diseases (for reviews, see 6,7,8,9,10,11). Furin requirements for efficient substrate hydrolysis depend not only on the well-established motif R-X-K/R-R but also on combinations of amino acids at P₅, P₆, P'₁, P'₂ and P'₃ substrate positions, and on the presence of potassium ions (K⁺), which activate the enzyme (12).

Measles virus infectivity in cultured cells has been reported to be increased by 200-fold in the presence of MgSO₄ (13). A similar effect for magnesium was reported for dengue virus in mammalian cell culture (14). Magnesium (Mg²⁺) is the second most abundant intracellular ion after K⁺; the free cytosolic Mg²⁺ concentration is about 1 to 3 mM (13,15,16) and is strictly controlled by specific transporters (reviewed by 17). In the present paper we report a detailed analysis of Mg²⁺ effects on furin activity using fluorescent resonance energy transfer (FRET) decapeptides derived from sequences that span the furin cleavage sites of viral envelope glycoprotein and eukaryotic proproteins. The virulence of influenza virus depends on its capacity to invade host cells, known to require haemagglutinin processing by a host proteinase; furin is the most accepted candidate enzyme for this processing event (for reviews 18,19). We here provide a detailed analysis of furin hydrolysis of peptides derived from H5N1 influenza hemagglutinin (HA). In order to evaluate whether Mg²⁺ can induce structural modifications in furin, the effects of Mg²⁺ on the pH-profiles of furin hydrolytic activity and on the intrinsic fluorescence were also examined.

Materials and methods

Enzymes

Recombinant human furin was expressed and purified as previously described (20) and the molar concentration of active enzyme was determined by active site titration with the decanoyl-RVKR-CMK inhibitor in 10 mM MES, 1 mM CaCl₂, pH 7.0 and the substrate Abz-GIRRKRSVSHQ-EDDnp. Secreted soluble Kex2 (ssKex2) was obtained from *S. cerevisiae*; this secreted form lacks the C-terminal tail and permits the production of enzyme in quantity for studies of specificity (21). Kex2 was purified from the culture media of the yeast strain mutant *S. cerevisiae* AFY490 transformed with the plasmid CB023-pG5KEX2 C3 and the gene URA3, which permits the cell division in the presence of uracil in the culture media. Kex2 was purified on a DEAE-Sepharose column (20 mL of resin). The resin was washed with 40mM Bis-tris buffer containing 10% Glycerol (v/v) at 4°C and the enzyme eluted in the same buffer containing 200 mM NaCl, concentrated in by Amicon ultrafiltration and stored at -80°C until used.

Peptides

All peptides were obtained by the solid-phase peptide synthesis strategy as previously described (22) using the Fmoc-procedure in an automated bench-top simultaneous multiple solid-phase peptide synthesizer (PSSM 8 system from Shimadzu, Tokyo, Japan). Details of purification and characterization have been reported earlier (12). Stock solutions of peptides were prepared in DMF and the concentrations were measured spectrophotometrically using the molar extinction coefficient of 17,300 M⁻¹cm⁻¹ at 365nm.

Kinetic measurements

The FRET peptides were assayed in 10 mM MES, 1 mM CaCl₂ and pH 7.0, using a HITACHI F-2500 spectrofluorimeter, at 37°C. The enzymes were pre-incubated in the assay buffer for 3 min before the addition of substrate. Fluorescence changes were monitored continuously at $\lambda_{\text{ex}}=320$ nm and $\lambda_{\text{em}}=420$ nm. When fluorogenic peptidyl-MCA substrates were used, the condition was changed to $\lambda_{\text{ex}}=380$ and $\lambda_{\text{em}}=460$ nm. The enzyme concentrations for initial rate determinations were chosen at a level intended to hydrolyze less than 5% of the amount of added substrate over the time course of data collection. The slope of the generated fluorescence signal was converted into micromoles of substrate hydrolyzed per minute based on a calibration curve obtained from the complete hydrolysis of each peptide. For each peptide the concentrations of the substrates for determination of their kinetic parameters were in the range of two times higher and lower of the obtained K_m value for furin hydrolysis. The kinetic parameters K_m and k_{cat} were calculated by non-linear regression using Grafit® software (Erithacus Software, Horley, Surrey, U.K.). Errors were less than 5% for each of the kinetic parameters obtained.

Each peptide product resulting from substrate hydrolysis was detected by its UV absorption at 220 nm and its molecular weight was determined by LC/MS using an LCMS-2010 equipped with a ESI-probe (Shimadzu, Japan) connected to the HPLC after the UV-detector. The HPLC conditions were: Ultrasphere C18 column (5 μm , 4.6 $\times$ 250 mm) which was eluted with the solvent systems A (H₃PO₄/water, 1:1000) and B (ACN/water/H₃PO₄, 900:100:1) at a flow rate of 0.8 ml/min and a 0–80% gradient of solvent B1 for 60 min.

Effects of MgCl₂ and pH on furin catalytic activity

The influence of MgCl₂ on the catalytic activity of human furin was tested in a concentration range of 1–100 mM; we also examined the effects of NaCl up to 100 mM under similar conditions. We measured the initial velocity of hydrolysis using 4 μM of the FRET substrates in 10 mM of MES buffer, 1 mM CaCl₂ at 37°C and pH 7.0.

The pH dependence of the various rate constants was measured at 37°C in a four-component buffer with constant ionic strength, comprised of 25 mM for acetic acid, MES and glycine and adjusted to the required pHs by the addition of 1M HCl or 1 M NaOH. The reaction was performed by assessing the V_{max} of the hydrolysis of substrates in the presence and absence of 5 mM MgCl₂. The enzyme was pre-incubated in buffer for about 2 minutes before starting to collect data. All the experiments were made under V_{max} rate constants where the substrate concentration was 3 times higher than its K_m . The values of V_{max} were fitted to equation 1 with the Grafit 5.0 software to theoretical curve for the bell-shaped pH-rate profiles using nonlinear regression.

$$V_{\text{max}} = V_{\text{max}}(\text{limit}) [1 / (1 + 10^{pK1 - \text{pH}} + 10^{\text{pH} - pK2})] \quad (\text{Eq. 1})$$

where, V_{max} stands for the pH-independent maximum rate constant and $K1$ and $K2$ are the dissociation constants of the catalytically competent base and acid, respectively.

Intrinsic fluorescence assays—The intrinsic fluorescence of the furin-containing solutions was obtained in a Hitachi-F2500 spectrofluorimeter with the excitation wavelength set at 280 nm (5 nm slit) and the emission scanned in the range of 300–400 nm (5 nm slit). The measurements were performed at 37°C, in 10 mM MES-NaOH buffer at pH 7.0; in the presence and absence of 1 mM CaCl₂ and 1 to 50 mM MgCl₂.

Results

Effects of Mg²⁺ on the hydrolysis of Abz-SRRHKRFAGV-Q-EDDnp by furin

The FRET peptide Abz-SRRHKR↓FAGV-Q-EDDnp contains the sequence of the Fo measles virus envelope glycoprotein with a furin cleavage site (↓) and also includes the donor and acceptor fluorescence groups Abz and Q-EDDnp, respectively. This peptide was used to investigate the effects of different Mg²⁺ concentrations on furin hydrolytic activity, as shown in Figure 1. A significant increase in the hydrolysis rate of Abz-SRRHKRFAGV-Q-EDDnp by furin was observed up to 5 mM Mg²⁺, at higher concentrations the rate of hydrolysis gradually decreased. KCl also increased the rate of hydrolysis of this peptide by furin, but NaCl had no effect. In the absence of Ca²⁺, furin was completely inactive, even in the presence of Mg²⁺ and K⁺ ions. The kinetic parameter K_m for the hydrolysis of Abz-SRRHKRFAGV-Q-EDDnp by furin decreased almost ten-fold at 10 mM Mg²⁺ and k_{cat} increased by a similar fold at 5 mM Mg²⁺ (Figure 2). These changes in K_m and k_{cat} resulted in a significant increase in the specificity constant k_{cat}/K_m, as depicted in Table 1 (peptide 32). The activation effects of Mg²⁺ and K⁺ ions on furin activity were additive for the hydrolysis of Abz-SRRHKRFAGV-Q-EDDnp when rates were determined at a fixed concentration of KCl (20 mM) under varying MgCl₂ concentrations (data not shown).

Effects of MgCl₂ on the pH profiles of furin activity

Figure 3 shows the pH profiles of furin hydrolysis for four FRET peptides in the presence and absence of MgCl₂. Due to the large effect of Mg²⁺ on its hydrolysis, the peptide Abz-SRRHKRFAGVQ-EDDnp was chosen as one of the substrates to evaluate the relationship of Mg²⁺ to the pH profile of furin activity. Abz-SRRHKRFAGVQ-EDDnp contains one H and its imidazole side chain has a pK that is in the pH range 6–7 (23). In order to exclude a substrate titration effect in the pH profile, we examined the hydrolysis of Abz-RKRSRRQVNTQ-EDDnp, which contains the sequence of the Ebola Sudan envelope glycoprotein and which is also highly activated by Mg²⁺ (peptide 1 in Table 1). We further examined the pH profiles of the hydrolysis of the peptide Abz-SGRSRRIDLQ-EDDnp from yellow fever virus M envelope glycoprotein, which contains a completely different prime site sequence, including a negatively charged D (peptide 10, Table 1). Lastly, the pH profile of the hydrolysis of the small substrate Ac-RVRR-MCA was also examined for comparison.

The optimum pHs for the hydrolysis of all four substrates were in the ranges 7.5–8.0 and 6.5–7.0 in the presence and the absence of MgCl₂, respectively. All of the data were fit to a single bell-shaped curve, and two pKs (pK₁ and pK₂) could be determined. The pK₁ values for the pH profiles of hydrolysis of the three substrates derived from virus envelope glycoprotein were similar in the presence and absence of MgCl₂, but the pK₁ was 0.5 units

higher in the presence of MgCl_2 for the peptide Ac-RVRR-MCA. The pK_2 values in the presence of MgCl_2 were higher than in the absence of salt for all four substrates. The pH-profiles for hydrolysis of substrates containing a positively charged imidazole of H, or a negatively charged carboxyl group of D in the prime sites, were very similar.

Intrinsic fluorescence

The intrinsic fluorescence spectra of furin in the presence of CaCl_2 and increasing amounts of MgCl_2 are shown in Figure 4. The fluorescence spectra are typical of W, and the presence of MgCl_2 did not shift the wavelength of the maximum peak of the spectra but significantly reduced the intensities of the peaks. The maximal fluorescence intensities from the five emission spectra obtained were plotted as a function of the MgCl_2 concentration. The fluorescence changes $F = F_0 - F_{\text{obs}}$, where F_0 is the initial fluorescence value of the furin solution and F_{obs} is the observed fluorescence value in the presence of MgCl_2 , fit to a single site saturation curve represented by equation (1)

$$\Delta F = \frac{\Delta F_{\text{max}} \cdot [M]}{K_{\text{d(obs)}} + [M]} \quad (\text{Eq 1})$$

The data fit to equation 2 with an observed dissociation constant ($K_{\text{d(obs)}}$) of $1.1 \pm 0.1 \text{ mM}$ for Mg^{2+} .

Effects of MgCl_2 on the hydrolysis by furin of FRET peptides derived from virus and human protein precursors

Tables 1 and 2 show the kinetic parameters of hydrolysis by furin in the presence and absence of MgCl_2 for the various FRET decapeptides (Abz-peptidyl-Q-EDDnp) derived from virus and human protein precursors. All of these peptides contain the canonical consensus cleavage site motif R-X-K/R-R required by furin, they are here grouped into the virus families from which the sequences of the envelope glycoproteins were derived. The peptides were ordered within each virus family by their relative $k_{\text{cat}}/K_{\text{m}}$ values (R) in the presence vs. the absence of MgCl_2 . In the presence of 5 mM MgCl_2 the $k_{\text{cat}}/K_{\text{m}}$ values for the hydrolysis of most of the FRET peptides derived from viruses were significantly higher than in the absence of Mg^{2+} (Table 1). In contrast, the $k_{\text{cat}}/K_{\text{m}}$ values for the hydrolysis of almost all FRET peptides derived from human proteins were largely reduced by Mg^{2+} (Table 2). These results indicate that the efficiency of Mg^{2+} in activating furin is highly dependent on particular combinations of amino acids at different substrate positions, a possible result of the cooperativity of furin subsites (12) and also observed in other proteases (recently reviewed in 24).

The haemagglutinin glycoprotein (HA) of the influenza A virus plays an essential role in viral invasion of the cells, which depends on its cleavage by host proteases into the fragments HA1 and HA2 (25). Such cleavage renders HA susceptible to conformational changes at low pH in the endosome (for reviews see 18,19). The FRET peptide Abz-RRRKKR↓GLFG-Q-EDDpp, which contains the processing site haemagglutinin glycoprotein (HA) of influenza A virus H5N1, required KCl for its hydrolysis by furin (Izidoro et al., 2009). Better substrates were obtained with analogues of this peptide

substituting amino acids that are more frequently found at prime site positions in substrates highly susceptible to furin (12). Table 3 shows the kinetic parameters for the hydrolysis of these peptides in the presence of Mg^{2+} . The FRET substrate Abz-RRRKKR↓GLFG-Q-EDDnp (peptide 44) was hydrolyzed only in the presence of Mg^{2+} , and the hydrolysis of analogues (peptides 45 to 47) modified at positions P_1' and P_3 with the amino acids most frequently found in the best substrates for furin (12) was also significantly activated by Mg^{2+} . It is noteworthy that highly efficient hydrolysis of the peptide Abz-RERRRKKR↓GLFG-Q-EDDnp (peptide 48) by Mg^{2+} -activated furin was observed; this peptide contains two additional residues (R and E) at its N-terminus. These two residues correspond to the two insertions reported in haemagglutinin in the avian influenza A (H5N1) virus obtained from a child with fatal respiratory illness in Hong-Kong (26).

Effects of $MgCl_2$ on the hydrolysis by Kex2 of FRET peptides derived from *Saccharomyces cerevisiae* pro- α -mating factor

Kex2 is the prototype of the large family of eukaryotic pro-protein processing proteases that includes furin (7); this enzyme is necessary for the production and secretion of mature α -mating factor and killer toxin in *S. cerevisiae* by proteolysis at paired dibasic sites (27). We examined the effects of Mg^{2+} on the hydrolysis by kex2 of Abz-YKR↓EAD-Q-EDDnp and Abz-LDKR↓EAE-Q-EDDnp, peptides containing kex2 cleavage sites derived from pro- α -mating factor. The k_{cat}/K_m values for the hydrolysis of these peptides by kex2 in the absence of Mg^{2+} were 7939 and 353 $mM^{-1}s^{-1}$, respectively. As shown in Figure 5, Mg^{2+} inhibited kex2-mediated hydrolysis of both peptides, not depending on the efficiently they were hydrolyzed in the absence of Mg^{2+} .

Discussion

The major finding of this work is that major and selective activation by Mg^{2+} ions of furin occurs for substrate sequences derived from virus envelope glycoproteins as compared to other eukaryotic proproteins. The peptide Abz-SRRHKR↓FAGV-Q-EDDnp (peptide 32, Table 1), derived from the measles virus fusion protein F_0 , exhibited a 60-fold increased k_{cat}/K_m value in the presence of Mg^{2+} . We hypothesize that the increase in yield of measles and dengue virus production in cell culture induced by $MgCl_2$ reported earlier (14,28) is related to the higher susceptibility of these virus envelope glycoproteins to furin cleavage in the presence of Mg^{2+} ions. The hydrolysis of peptides derived from the filoviridae (Ebola) and herpesviridae virus families were also significantly increased by $MgCl_2$. The especially large activation by Mg^{2+} of the hydrolysis of the peptide Abz-RERRRKKR↓GLFG-Q-EDDnp (peptide 48, Table 3) derived from Asian avian influenza A (H5N1) is noteworthy. As the pathogenicity of influenza virus depends on its capacity to invade the host cells, which is in turn dependent on host proteinase processing of virus haemagglutinin, the insertion of the amino acids E and R in this particular substrate possibly renders its haemagglutinin more susceptible to furin hydrolysis in the presence of cellular Mg^{2+} ions- thus greatly improving virus infectivity.

The efficiency of furin hydrolysis was highly dependent on the particular combinations of amino acids at different substrate positions, as previously reported (12). Similar

interdependence of furin subsites also seems to occur in the activation of furin by MgCl_2 , and it is very possible that a large degree of cooperativity between furin subsites exists as recently reported for other peptidases (24,29). A more detailed analysis of cooperativity among the various furin subsites is clearly required; this work is currently in progress in our laboratory.

Any analysis of cooperativity should take into account that all furin substrates are highly positively charged; the accommodation of guanidinium and ammonium side chain cations at the negatively charged S_1 , S_2 and S_4 furin subsites can most likely be modulated by the occupancy of the other subsites and by salts. In addition, activation by Mg^{2+} seems to be a furin peculiarity that we did not observe with kex2, the prototype of the convertase family which includes furin.

Although the free concentrations of Mg^{2+} in cell compartments are not known, in most mammalian cells this free ion is present in the range of 1 to 3 mM and represents less than 10% of the total magnesium within cells (13,15,16). It is noteworthy that the $K_D = 1.08 \pm 0.1$ mM for the dissociation constant of Mg^{2+} from furin, obtained with intrinsic fluorescence measurements, is within the physiological range of intracellular Mg^{2+} concentrations. However, we should consider that cells infected by virus can have Mg^{2+} transport through the cell membrane affected in a manner that results in increased Mg^{2+} intracellular accumulation.

Any proposal to explain the mechanism of activation of furin by cations would be highly speculative at this point and would also require further information on the interdependence of furin subsites. However, it seems probable from the data presented above that virus envelope glycoprotein mutations have been selected to increase susceptibility to furin inside cells, a milieu rich K^+ and Mg^{2+} ions. An example of this process is likely to be the insertion of the amino acids E and R in the H5N1 influenza virus hemagglutinin, which greatly increases virulence, possibly by increased susceptibility of this sequence to Mg^{2+} -stimulated furin hydrolytic activity.

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Abbreviations

ADAM-TS	A Disintegrin and Metalloproteinase with Thrombospondin
hBMP	Human Bone Morphogenetic Protein
MT-MMP	Membrane Type-Matrix Metalloprotease
TGF-β	Transforming Growth Factor - β
HHV	Human Herpes Virus
CMV/AD169	Cytomegalovirus/strain AD169-protein

CMV/Towne	Cytomegalovirus/strain Town-protein
MCA	amino-(methyl)-coumarin
FRET	Fluorescent Resonance Energy Transfer
PACE	Paired Basic Amino Acid Cleaving Enzyme
ACN	acetonitrile
DTT	dithiothreitol
Abz	<i>ortho</i> -aminobenzoic acid
EDDnp	<i>N</i> -(2,4-dinitrophenyl) ethylenediamine
TGR resin	(dimethoxybenzhydramine-PEG)-resin
tBu	<i>tert</i> -butyl
Fmoc	(fluoren-9-ylmethoxycarbonyl)
HOBt	N hydroxybenzotriazole
HBTU	O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate
EDT	ethanedithiol
TFA	trifluoroacetic acid

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Figure 1.

Effects of MgCl_2 (○), KCl (●) and NaCl (□) on the hydrolysis of the FRET peptide Abz-SRRHKRFAGVQ-EDDnp (peptide 1; 4 μM) by furin. Kinetics were examined in buffer containing 10 mM MES, 1 mM CaCl_2 , 0.01% Triton X-100, pH 7.0 at 36.5 °C. (▲) represents the kinetic conditions in the absence of 1 mM CaCl_2 but in the presence of 5mM MgCl_2 , 20 mM KCl.

Figure 2. Effects of MgCl₂ on the kinetic parameters k_{cat} (○) and K_{m} (●) for the hydrolysis of Abz-SRRHKRFAGVQ-EDDnp (peptide 1) in 10 mM MES, 1 mM CaCl₂, 0.01% Triton X-100, at pH 7.0 and 36.5 °C.

Figure 3.

pH-profile of furin activity and effect of $MgCl_2$ in the presence (●) and absence (○) of 5 mM $MgCl_2$. pKs in the presence of $MgCl_2$ (values in parentheses are without KCl).

Abz-RKRSRRQVNTQ-EDDnp - pK₁ = 5.46 (5.67) and pK₂ = 9.54 (7.80)

Abz-SRRHKRFAGVQ-EDDnp - pK₁ = 5.27 (5.42) and pK₂ = 9.54 (8.93)

Abz-SGRSRRAIDLQ-EDDnp - pK₁ = 5.70 (6.35) and pK₂ = 9.10 (8.00)

Ac-RVRR-MCA - pK₁ = 7.10 (6.41) and pK₂ = 9.20 (8.60)

Figure 4.

Effect of magnesium chloride on the intrinsic fluorescence of furin. Spectra of furin without salt (—), in the presence of 1 mM (— — —), 5 mM (.....), 30 mM (— · —) and 50 mM (— · · —) MgCl₂. The insert shows the binding curve of magnesium chloride for furin ($K_d = 1.08$ mM).

Figure 5. Effects of Mg^{2+} on the hydrolysis of 12 μM of Abz-YKREAD-Q-EDDnp (●) and 130 μM of Abz-LDKREAE-Q-EDDnp (○) by Kex2. The experiment was performed using 200 Bis-Tris, 1 mM CaCl_2 , 0.01% Triton and pH 7.0 at 36.5°C.

Table 1

Kinetic parameters for the hydrolysis by human furin of FRET peptides (Abz-peptidyl-Q-EDDnp) derived from the sequences of viral envelope glycoproteins in the presence of 5 mM MgCl₂. Shown in parentheses are the values in the absence of MgCl₂. Conditions of hydrolysis: Mes 10 mM, 1 mM CaCl₂, 0,01% Triton X-100, pH 7.0 and 36.5°C. Errors were less than 5% for each of the kinetic parameters. R: relative k_{cat}/K_m values in presence vs. absence of MgCl₂

	Abz-peptidyl-Q-EDDnp	k _{cat} (s ⁻¹)	K _m (μM)	k _{cat} /K _m (mM.s) ⁻¹	(R)
Filoviridae					
1	RKRSRR↓QVNT (Eb. Sudan GP)	11.3 (8.4)	0.20 (6.5)	56300 (1291)	44
2	QIRAKR↓ELSK (Eb. Reston sGP)	6.7 (9.7)	1.2 (12)	5583 (808)	6.9
3	GRTRR↓EAIV (Eb. Zaire GP)	4.2 (3.6)	0.21 (1.2)	20190 (3085)	6.5
4	TRKQKR↓SVRQ (Eb. Reston GP)	9.3 (9.8)	1.6 (10.6)	5819 (918)	6.3
5	VVRVRR↓ELLP (Eb. Zaire sGP)	16.6 (12.2)	1.3 (3.5)	13071 (3466)	3.8
6	SRRKRR↓DVTP (Eb. Ivory Coast GP)	9.9 (9.2)	0.15 (0.45)	66000 (20467)	3.2
7	MMRHR↓ELQR (Eb. Sudan sGP)	10.3 (16.2)	0.44 (1.2)	23409 (13508)	1.7
8	LTRQRR↓SLLP (Eb. Ivory Coast sGP)	3.3 (1.8)	0.9 (0.64)	3510 (2708)	1.3
9	YFRKRR↓SILW (Marburg Virus GP)	35.0 (28.6)	13.0 (5.6)	2692 (5124)	0.5
Flaviviridae					
10	SGRSRR↓AIDL (Yellow Fever virus M)	6.2 (4.3)	0.39 (0.81)	15923 (5328)	3.0
11	GSRTTR↓SVLI (Tick-borne Encephalitis)	7.2 (3.7)	1.4 (1.5)	5091 (2460)	2.1
12	SRRSRR↓SLTV (West Niles virus M)	6.2 (4.3)	0.39 (0.81)	15923 (5328)	1.9
13	SKRSRR↓SVSV (Japan β Encephalitis)	18.3 (10.5)	0.49 (0.41)	37347(25362)	1.5
14	HRQKR↓SVAL (Dengue 3)	14.9 (9.2)	0.37 (0.28)	40270 (32431)	1.2
15	RRDKR↓SVAL (Dengue 4)	12.2 (14.6)	0.41 (0.39)	29658 (37505)	0.8
16	HRREKR↓SVAL (Dengue 2)	7.6 (12.2)	0.20 (0.22)	38300 (55636)	0.7
17	HRDKR↓SVAL (Dengue 1)	12.7 (9.5)	0.55 (0.26)	23091 (36615)	0.6
Retroviridae					
18	VQREKR↓AVGI (HIV GP-160)	2.7 (1.2)	2.1 (2.8)	1290 (407)	3.2
19	GIRRK↓SVSH (Rous Sarcoma GP)	29.6 (30.3)	0.42 (0.39)	70547 (77100)	0.9
20	SIRHKR↓EPSV (Friend Leukemia)	1.6 (2.1)	2.4 (2.8)	646 (729)	0.9
21	KRRQRR↓RPPQ (HIV-1 Tat)	Slow Hydrolysis			

	Abz-peptidyl-Q-EDDnp	kcat (s ⁻¹)	Km (μM)	kcat/Km (mM.s) ⁻¹	(R)
Herpesviridae					
22	SRRKRR↓SAST (HHV-8)	11.1 (10.0)	0.14 (5.0)	76232 (1992)	38
23	HNRTKR↓STDG (CMV/Towne)	7.8 (0.26)	0.95 (0.87)	8210 (289)	28
24	THRTRR↓STSD (CMV/AD169)	8.1 (0.44)	1.9 (1.2)	4128 (370)	11
25	LRRRRR↓DAGN (Epstein Barr gp 110)	12.3 (1.1)	3.6 (3.3)	3455 (348)	9.9
26	KIRRRR↓DVVD (HHV-6A)	3.1 (0.60)	0.05 (0.10)	53448 (6000)	8.9
27	RKRRKR↓ELET (HHV-7)	4.3 (0.23)	3.1 (1.4)	1400 (164)	8.5
28	NLRRRR↓DLVD (HHV-6B)	3.5 (2.0)	0.33 (1.4)	10606 (1470)	7.2
29	NTRSRR↓SVPV (Varicella Zoster Gb)	Slow Hydrolysis			
Orthomyxoviridae					
30	LKRRRR↓DTQQ (Borna Disease)	6.7 (0.61)	5.2 (0.28)	1303 (2184)	0.6
31	RRKKR↓GLFG [Influenza HA (H5N1)]	5.0 (NH)	15.5 (NH)	322 (NH)	-
Paramyxoviridae					
32	SRRHKR↓FAGV (Measle virus Fo)	2.4 (0.21)	0.07 (0.37)	33857 (559)	60
33	KKRKKR↓FLGF (Respiratory-Syncytial virus)	1.1 (0.64)	0.52 (1.3)	2173 (512)	4.2
Coronaviridae					
34	TRRRR↓SITE (Infectious Bronchitis)	19.8 (16.4)	0.53 (0.58)	37358 (28362)	1.3

Kinetic parameters for the hydrolysis by human recombinant furin of FRET peptides (Abz-peptidyl-Q-EDDnp) derived from the sequences spanning the cleavage sites for the activation of human proproteins in the presence of 5 mM MgCl₂. The values in parentheses were obtained in the absence of MgCl₂.

Table 2

	Abz-peptidyl-Q-EDDnp	k _{cat} (s ⁻¹)	K _m (μM)	k _{cat} /K _m (mM.s) ⁻¹	(R)
35	RRRAKR↓SPKH (hBMP-4 (1st site)	5.3 (8.7)	1.2 (12.2)	4383 (710)	6.2
36	SSRHRR↓ALDT (hTGF-β3)	7.1 (5.6)	4.16 (0.86)	1707 (6488)	0.3
37	HHRQRR↓SVSI (hADAM-TS 6)	28.9 (39.5)	3.2 (0.3)	9031 (116088)	0.1
38	Ac-RVRR-MCA	0.59 (0.98)	4.9 (0.86)	120 (1139)	0.1
39	HKREKR↓QAKH (hBMP-2 (1st site)	6.3 (6.9)	2.7 (0.38)	2354 (17734)	0.1
40	GQRKKR↓ALDT (hTGF-β2)	3.6 (3.9)	4.4 (0.27)	829 (14130)	0.06
41	QSRRRR↓QTPP (MT-MMP 4)	3.4 (6.3)	2.21 (0.20)	1547 (31067)	0.05
42	RNRQKR↓FVLS (MT-MMP 3)	NH (0.20)	NH (0.59)	NH (337)	-
43	VRRRRR↓YALS (MT-MMP 6)	NH (0.60)	NH (3.14)	NH (193)	-

Conditions of hydrolysis are the same as in Table 1. Ac-RVRR-MCA was included for comparison

Kinetic parameters for the hydrolysis by human recombinant furin of FRET peptides derived from Abz-RRRKKRGLFG-Q-EDDnp, the H5N1 influenza hemagglutinin (HA) processing site, in the absence (in parentheses) and in the presence of 5 mM MgCl₂.

Table 3

N ^o	Abz-peptidyl-Q-EDDnp	kcat (s ⁻¹)	Km (μM)	kcat/Km (mM.s) ⁻¹	(R)
44	RRRKKR↓GLFG [Influenza HA (H5N1)]	5.0 (NH)	15.5 (NH)	322 (NH)	-
45	RRRKKR↓GLSG (F>S)	6.7 (2.4)	0.22 (3.01)	30500 (797)	38
46	RRRKKR↓SLFG (G>S)	7.6 (1.0)	0.23 (1.65)	33043 (606)	54
47	RRRKKR↓SLSG (G>S, F>S)	4.5(0.92)	0.24 (0.69)	18833 (1333)	14
48	RERRKKR↓GLFG [Influenza HA (H5N1)]	12.3(2.6)	0.1(1.6)	123000 (1555)	79