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The Potential Roles of Cell Surface pHs in Bioactive Peptide Activation

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Abstract

Glycolytic metabolism of cells produces protons that are removed from the cytosol by transport proteins to create a pH difference between the adjacent bulk solution and the cell membrane surface. Therefore, tissue cells have distinct surface pHs because of varied glycocalyx and proton production capability. In this study, we proved the role of cell surface pH in peptide-cell interaction and peptide activation by using lytic peptides with pH-dependent activity as probes. Properly selected peptides could sense the specific pH zones on cells and thus demonstrated varied activity to tissue cells with different surface pHs. For a specific cell, the activity of pH-sensitive peptides changed accordingly as the cell surface pH was tuned up or down by proton channel regulators. Mechanistic studies revealed that cell surface pH directly affected peptide insertion into membranes by altering the secondary structure and aggregation status of membrane bound pH-sensitive peptides. A pH-sensitive lytic peptide-designed based on the cell surface pH difference between a normal-cancer cell pair showed good selectivity to cancer cells. Therefore, cell surface pHs may present new opportunities to design therapeutic peptides with high cell specificity and selectivity.

Keywords

Cell surface pH; pH sensitive; peptide; self-assembly; aggregation

INTRODUCTION

The glycolytic metabolism of cells produce protons that are removed from the cytosol by transport proteins such as the Na⁺/H⁺ exchanger isoform 1 (NHE1).^{1,2} Without constraints on proton diffusion, the ejected protons will be diluted in the infinite external solution. However, the surface of mammalian cells is covered by a dense layer of carbohydrates,

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CONFLICT OF INTEREST

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which is composed of the conjugated oligosaccharide chains of the membrane-anchored glycoproteins and glycolipids, called the glycocalyx.³ Because of the presence of these negatively charged macromolecules, the diffusion of protons across the membrane/water interface is indeed restricted by the low dielectric permittivity (ϵ) of water at the negatively charged membrane surfaces. As a result, the ejected protons readily spread over the cell membrane surface but are somehow prevented from prompt equilibration with the bulk. It is estimated that this potential barrier can raise the proton concentration at the membrane surface by 10^{-6} M over the value in the bulk, creating the pH difference between cell membrane surfaces and the adjacent bulk solution.^{4,5} Theoretically, this specific pH zone on cell surfaces could have great impact on cells by affecting cell surface charges, ion accumulation on cell surfaces, cell membrane potentials, drug uptakes, and peptide/protein binding to receptors. Unfortunately, the biological importance and potential pharmaceutical significance of cell surface pHs have been overlooked.

We know that peptide transition into the plane of binding and insertion into cell membranes are critical steps for bioactive peptides to exert their biological activities.⁶ In this study, a group of lytic peptides with pH-dependent cell lysis activity (pH-sensitive lytic peptides) were selected as probes to evaluate the pharmaceutical significance of cell surface pHs by examining cell surface pHs affected peptide-cell interactions and peptide activation.

MATERIALS AND METHODS

Materials

Peptides (>90% in purity) were synthesized by Genescript Corp. (Piscataway, NJ). Peptides were dissolved in dimethyl sulfoxide (DMSO) to form 5.0 mM stock solutions. Peptide working solutions were prepared from the stock solution by gradual dilution using proper cell culture medium. LIVE/DEAD bacteria staining kit was purchased from Invitrogen Life Technologies (Carlsbad, CA). All other chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO).

Cell cultures

All cells were obtained from American Type Culture Collection (ATCC). A549 and CHO-K1 cells were grown in F12K, NIH/3T3 cells in DMEM, and CCD-Lu13 cells in MEM. All mediums were supplemented with 10% Fetal Bovine Serum (FBS). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO₂.

The pH Sensitivity of Peptides

The lytic activity of peptides was tested on calcein loaded large unilamellar vesicles (LUVs) as described previously.⁶ Peptide-induced calcein leakage, reflected by an increase in fluorescence intensity, was monitored using a fluorescent Microplate Reader by setting the excitation and emission wavelengths at 485 nm and 530 nm respectively. Calcein release from LUVs was represented as F/F_0 , where F_0 and F represent the fluorescence intensity of calcein loaded LUVs in the absence and presence of peptides, respectively. It has proven that the activity change of pH-sensitive lytic peptide usually happens in a narrow pH range at the so-called peptide transition pH, a pH point when the peptide had zero net charge,⁶ as

shown in Figure 1. The transition pHs of peptides were determined using Nanosizer.⁶ Briefly, peptides from stock solutions (5 mM in water) were diluted with PBS of various pHs (pH=5.0~9.0). Freshly prepared peptide solutions (40 μ M) were subjected to a brief (60 second) sonication treatment. The Zeta potentials of the peptide solutions were measured immediately after sonication using a Zeta nanosizer.

Measurement of cell surface pHs

Total 2.5×10^5 cells were seeded on collagen-coated cover glass and cultured overnight. Cells were stained with fluorescein conjugated wheat germ agglutinin (WGA) (12.5 μ M) in serum free medium for 30 minutes, and then the cover glass was assembled to a FCS2 flow chamber system (Biopetechs Inc.). The pH was measured using video imaging techniques. The cover glass was placed on the stage of a confocal microscope (LSM510; Carl Zeiss, Inc.) and continuously superfused with pre-warmed (37 °C) Hepes-buffered Ringer's solutions (122.5 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl₂, 1.2 mM CaCl₂, 1.0 mM NaH₂PO₄·2H₂O, 5.0 mM glucose, 10 mM Hepes; pH=7.4) at 0.1 ml/min. The excitation wavelength alternated between 458 nm and 488 nm while the emitted fluorescence intensities were recorded beyond 560 nm. A ratio was calculated from emitted fluorescence intensities measured at 458 and 488 nm. Thus, the measurement was virtually independent of the amount of dye excited in a given region of interest and represented the local proton concentration.¹ Fluorescence intensities were measured at 1.0 second intervals and averaged 10 times, and corrected for background fluorescence by subtracting background intensities of control regions placed right next to the measured regions of interest. In the end of each experiment, the pH measurements were calibrated by successively superfusing the cells with modified Ringer's solutions (125 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 20 mM Hepes, and 10 μ M nigericin) of varied pHs (pH=10, 9, 8.5, 8.0, 7.4, 7.0, 6.5, and 6.0). Fluorescein had a good fluorescence intensity-pH correlation in the pH range of 6–10.

Determination of cell surface charges

Cells were seeded in cell culture flasks at a concentration of 5×10^4 cells/ml. After 48 hr incubation, cells were scraped off from culture flasks, washed with PBS, and re-suspended in 0.25 M sucrose solution to a final concentration of 10^6 cells/ml. The cell suspension was then stained with 1% Toluidine Blue for 90 minutes. After that, cells were titrated with poly (1,1-dimethyl-3,5-dimethylenepiperidinium chloride) (CFC) solution. Cell surface charges were calculated by normalizing total charges against cell numbers according to the procedure described in previous publications.⁷

Acquisition of peptide circular dichroism (CD) spectra

The CD spectra of peptides were recorded on a Jasco J-710 spectropolarimeter.⁶ The CD spectra were scanned at 25 °C in a capped, quartz optical cell with a 1.0 mm path length. Data was collected from 250 to 190 nm at an interval of 1.0 nm with an integration time of two seconds at each wavelength. Five to ten scans were averaged, smoothed, background-subtracted, and converted to mean residue molar ellipticity $[\theta]$ (degrees cm² dmol⁻¹) for each measurement. CDPRO software was used to analyze the data obtained from the CD spectropolarimeter.

Measurement of peptides induced cell lysis

Overnight cultured cells on 96-well plates (5×10^3 cells/well) were washed with PBS three times before being exposed to peptides. Peptide-induced cell membrane damage was quantitatively assessed by measuring lactate dehydrogenase (LDH) release from damaged cells using the LDH kit after 60 minutes of incubation. The absorbance at 490 nm was measured using a microplate reader (Biotek Inc.) by setting a reference wavelength at 690 nm. Results were normalized to cell death percentage against both blank and 100% lysis controls.

Dynamics of peptide-induced cell membrane damages

Freshly trypsinized human lung carcinoma A549 cells and were seeded in a collagen-coated 8-well glass chamber (2×10^4 cells/well) and cultured overnight. Before the assay, cells were washed with PBS three times and stained with the LIVE/DEAD staining kit for 15 min. After the addition of peptides, cell images were recorded at different time points using a Zeiss LSM510 Confocal Microscope. The excitation wavelength was fixed at 488 nm, and the emission wavelengths were set at 505–530 nm (for the live cells) and 560 nm (for the dead cells), respectively. The percentage of green pixels out of total green and red pixels in captured cell images was calculated to estimate the cell membrane integrity.⁸

Peptide interactions with lipid membranes

Peptide interactions with cell membranes were studied using a lipid monolayer assembled on the Micro Trough X (Kibron Inc.).⁶ A lipid mixture of DPPC/Cholesterol/DPPS (50/10/2.5) was dissolved in 3:1 chloroform:methanol to form 100 μ M lipid solutions. To a four-well Teflon plate, 1.0 mL PBS was added, followed by a drop of lipid solution. A lipid monolayer was formed on the water surface after the evaporation of organic solvent. Peptide-induced surface tension change was recorded right after peptide solutions were added to the subphase through the side pores of the Teflon plate.

Toxicity of pH-sensitive peptides to normal and cancer cells

The cytotoxicity of pH-sensitive and pH-insensitive lytic peptide to normal and cancer cells were compared on a cancer-normal pair (A549 and CCD-13Lu) using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, cells in complete medium were added into 96-well plates (5×10^3 cells/well) and cultured at 37 °C for 14–16 hours. After being washed, cells were fed with serum-free medium containing various concentrations of peptides and incubated at 37 °C for 2 hours. After that, 10 μ L of MTT (5 mg/mL) was added into each well. Cell viability was determined after 4 hours of incubation by dissolving formazan with 10% SDS solution containing 5% isopropanol and 0.1% HCl and measuring absorbance at 570 nm.⁹

Characterization of peptide self-assembly and aggregation in solutions

Peptide self-assembling in solutions to form peptide aggregates was estimated using a fluorescence probe 1-anilinonaphthalene-8-sulfonic acid (1, 8-ANS).^{6,10} 1, 8-ANS (20 μ M) fluorescence emission spectrum increase at 460–500 nm caused by peptide aggregation was

recorded on a fluorescent Microplate Reader (Biotek Inc.) by setting excitation wavelength at 369 nm.

Particle size and zeta potential change of peptide solutions at different pHs were measured using a Zeta nanosizer. Peptide solutions were subjected to a brief (60 second) sonication treatment right before each measurement.

Morphologies of peptide aggregates were studied using scanning electron microscopy (SEM). Peptide solutions were loaded onto silicon wafers and incubated for 30 minutes. After washing with deionized water, peptide samples on silicon wafers were coated with gold. SEM images were acquired using Auriga Modular CrossBeam workstation (Carl Zeiss Inc., Thornwood, NY).

RESULTS AND DISCUSSION

Selection of cells and probing peptides

Although extracellular environments have impact on cell surface pH by affecting proton exchange between cell surfaces and bulk solutions, under physiological conditions with fixed pH, cell surface pH will be determined by the chemical and biological properties of the cells including proton production capacity of a cell, cell size, and the pH-buffering capacity of cell surfaces. Therefore, tissue cells may have specific and even distinguished surface pHs under physiological conditions. We measured the cell surface pHs of a group of cells from different organs/tissues at physiological pH and found that all tested cells had distinct surface pHs (Table 1).

Lytic peptides are a group of cationic peptides which have a primary target on the cell membrane and kill cells by causing cell lysis. Studies on lytic peptide-cell interactions have revealed that transition of peptides into the plane of binding and insertion into membranes are critical for lytic peptides to induce cell membrane lysis.^{6, 9, 11} We chose lytic peptides with pH-dependent cell lysis activity as probes to investigate the potential effects of cell surface pHs on peptide-cell interactions. If pH-sensitive lytic peptides could sense the pH difference between the bulk solution and cell surfaces to have changed peptide-cell interactions and show altered cell lysis activity, the potential pharmaceutical significance of cell surface pHs could be assessed.

We have developed a general approach to construct lytic peptides with pH-dependent cell lysis activity.^{9,12} Some pH-sensitive lytic peptides demonstrate up to 30 fold activity increase in response to decreased medium pHs.¹² Because electrostatic attraction of peptides to cell membranes is the first and also a critical step to bring peptides to cell surfaces, net charges on peptides will have dramatic effects on peptide-cell interactions. In this regard, only pH-sensitive lytic peptides with the same (+1) charges (Table 2) were selected. In addition to charges, the secondary structure of peptide may also dramatically affect peptide-cell interactions. The folding of the peptide chain into a specific conformation, normally an alpha-helical structure, proves to be a necessary and energy favorable process for peptide insertion into cell membranes.^{6,13} In this study, pH-sensitive lytic peptides with the same

secondary structure (α -helix) were selected. Such peptide and cell selection enabled us to directly compare results obtained from different cells and peptides.

Peptide CL-1 is a pH-insensitive lytic peptide (Table 2). Despite its sequence and structure similarity with other pH-sensitive peptides, the cell lysis activity of the peptide was hardly affected by pHs. Peptide CL-1 was used as a negative control in this study.

Activities of pH-sensitive lytic peptides to cells with different surface pHs

Activities of pH-sensitive lytic peptides were first tested on CHO-K1 cells with a measured cell surface pH=8.40. Because the surface pH of CHO-K1 was much higher than the transition pHs of all selected peptides (Table 2), all peptides would carry the same negative charges as they translocated to cell surfaces from the bulk solution (pH=7.4). Four tested pH-sensitive lytic peptides demonstrated low and nearly the same cell lysis capability on CHO-K1 cells (Figure 2).

In order to investigate the potential effect of cell surface pH on pH-sensitive lytic peptide interactions with cells, we used a proton channel regulator, 8-CPT-cAMP,¹⁴ to tune cell surface pHs. Under normal cell culture conditions, 8-CPT-cAMP had no effects on the viability of CHO-K1 cells (Figure 2). However, the cell surface pH of CHO-K1 was quickly (within 30 minutes) reduced to pH=6.90 from pH=8.4 by 8-CPT-cAMP while the medium pH was kept unchanged at pH=7.4 (Table 3). Although the surface pH of 8-CPT-cAMP treated CHO-K1 cells was still higher than the transition pHs of peptide CL-9 (pH=6.80) and CL-10 (pH=6.60), it was lower than the transition pHs of peptide CL-7 (pH=7.35) and CL-22 (pH=7.15). Interestingly, four pH-sensitive lytic peptides demonstrated varied cell lysis ability to 8-CPT-cAMP treated CHO-K1 cells: peptide CL-9 and CL-10 maintained the same activity as they were on untreated CHO-K1 cells while peptide CL-7 and CL-22 showed dramatically increased (about two folds) cell lysis activity (Figure 2). The activity results of peptides matched the surface pHs on untreated and 8-CPT-cAMP treated CHO-K1 cells, suggesting the involvement of cell surface pH in pH-sensitive peptide activation.

In order to exclude the possibility that the above results were caused by some intrinsic properties of CHO-K1 cells, we performed the same experiment on another selected cell, A549, with nearly identical cell surface charges as CHO-K1 cells (Table 1). Because peptides would be subjected to the same pulling force from A549 and CHO-K1 cells, potential effects of electrostatic attractions on peptide-cell interactions were minimized or eliminated. A549 cells had a surface pH=7.15 which was higher than the transition pHs of peptide CL-9, CL-10, and CL-22, but lower than the transition pH of peptide CL-7 (pH=7.35). Again, CL-7 proved to be the most active peptides on A549 cells (Figure 3). In the presence of 8-CPT-cAMP, the surface pH of A549 cells was reduced to pH=6.55 (Table 3) which was lower than the transition pHs of all tested lytic peptides (Table 1). As a result, four pH-sensitive lytic peptides showed dramatically increased cell lysis activity to 8-CPT-cAMP treated A549 cells (Figure 3). It should be noted that unlike pH-sensitive peptides, the activity of CL-1, a pH-insensitive peptide, to A549 and CHO-K1 cells hardly changed as cells were treated by 8-CPT-cAMP (Figure 2 & 3).

Further evidence that cell surface pHs affected peptide activation were from similar studies using another proton channel regulator, Bafilomycin A1.¹⁵ Like 8-CPT-cAMP, Bafilomycin A1 had very limited effects on cell viability (Figure 4) but Bafilomycin A1 treatment led to increased cell surface pHs. In addition, Bafilomycin A1 was able to reverse 8-CPT-cAMP's effect on cells and brought cell surface pHs back to the normal levels or beyond (Table 3). Studies revealed that the elevated cell lysis activities of peptide originally observed on 8-CPT-cAMP treated cells were reversed as the cell surface pH was increased beyond the transition pHs of peptides by Bafilomycin A1 (Figure 4).

Mechanism study

Peptide and cell membrane interactions include three steps: 1) electrostatic attraction of cationic peptides to cell surfaces; 2) the transition of the peptide into the plane of binding; and 3) the insertion of peptides into cell membranes. Details on peptide-cell interaction were investigated by studying the dynamics of pH-sensitive peptide CL-7 induced membrane damages (Figure 5A). CL-7 induced cell membrane leakage on A549 cells was time dependent and followed a linear curve. Although CL-7 mediated cell membrane damage on 8-CPT-cAMP treated A549 cells followed the same curve at the beginning, it deviated from the linear curve and accelerated after 15–20 minutes incubation (Figure 5A). A parallel membrane tension study performed on a lipid monolayer membrane revealed that CL-7 interactions with cell membranes were characterized by an initial phase of rapid surface tension increase followed by a second phase of surface tension relaxation (Figure 5B) which correlated with peptide binding to membranes and peptide insertion in membranes, respectively. Although CL-7 binding to lipid membranes was a fast process (3–5 minutes), peptide insertion into lipid membranes took about 20 minutes to complete. Results from these two experiments matched very well, implying that the dramatic effect of cell surface pH on CL-7 occurred during the late phase of peptide-cell interaction, i.e. peptide insertion in cell membranes.

We know that histidine-containing pH-sensitive peptides tend to aggregate and can self-assemble into peptide aggregates with defined structures because of reduced net positive charges on peptides.^{8, 9, 16} Peptides with altered aggregation at different pHs is a major reason for the pH-dependent activity of histidine-containing peptides.^{6, 9} Studies using 1, 8-ANS as a probe to the hydrophobic “pockets” of peptide aggregates (Figure 6A) revealed that peptide CL-7 underwent self-assembly at physiological pH as indicated by the high emission intensity and significant blue shift of 1, 8-ANS peak at 500 nm. CL-7 aggregates composed of fibril-like super-molecules and nano-sized particles were visualized using SEM (Figure 6B). However, like other histidine-containing peptides, CL-7 aggregates were unstable and dissolved under acidic conditions as the imidazole groups of histidine residues were protonated and the intermolecular repulsions among peptide molecules were resumed (Figure 6A). This pH controlled CL-7 aggregation and dissolution were confirmed through particle analysis conducted in high concentration (80 μ M) peptide solution (Figure 6C). Peptides in the free form will have increased membrane affinity and membrane insertion ability compared to peptide aggregates,^{6, 9} which explains the strong activity of pH-sensitive lytic peptides to cells with acidic surface pHs (Figure 2, 3, & 4).

In addition to peptide aggregation status, cell surface pH may also affect the secondary structure of pH-sensitive peptides. It has been found that peptides insertion into cell membranes is usually accompanied by a peptide secondary structure change¹⁷. The folding of the peptide chain into a specific conformation, normally an α -helical structure, proves to be a necessary process for peptide insertion into cell membranes.⁶ Although all selected pH-sensitive lytic peptides adapted α -helical structure at physiological pH, they changed into random coils as environment pHs dropped below the transition pHs of peptides (Table 2 and Figure 7). Changing from random coil to helix is an energy favorable process for peptide insertion into membranes.⁶ Thus, cell surface pH may also affect the activity of pH-sensitive peptides by altering their secondary structures.

Selectivity of pH-sensitive peptides to cancers

Involvement of cell surface pHs in peptide activation suggest a new opportunity to design therapeutic peptides with desired cell selectivity. We know that cancer cells usually have elevated metabolism and high cell surface buffering capabilities.¹⁸ Therefore, cancer cells may have more acidic surface pHs compared to their normal counterparts. Between a normal-cancer cell pair included in our studies, CCD-13Lu and A549, human lung carcinoma cell A549 had a more acidic surface pH (pH=7.15) than that of the normal human lung cell CCD-13Lu (pH=7.4) (Table 1). Despite such a small surface pH difference, it did provide us the chance to test the feasibility of utilizing cell surface pHs for improved cancer therapy.

A well studied pH-sensitive lytic peptide PTP-7b (FLGALFKALSHLL)^{6, 9} was selected in the cytotoxicity study. Because transition pH (pH=7.25) of PTP-7b was lower than the surface pH of A549 but higher than that of CCD-13Lu, the lytic peptide PTP-7b could be more toxic to A549 than to CCD-13Lu cells. The parent peptide of PTP-7b, PTP-7 (FLGALFKALSKLL), a pH-insensitive lytic peptide^{6, 9} was selected as a control. As shown in Figure 8, although peptide PTP-7 was equally active to both cells, pH-sensitive peptide PTP-7b was about three times more toxic to A549 than to CCD-13Lu.

We know that peptides have some unique and superior features compared to proteins.¹⁹ Bioactive peptides have a positive impact on the functions and conditions of living organisms and show several useful properties for human health. Over the last decade, there has been a rapid expansion in the study of peptides, and this is likely to continue. The unique and specific cell surface pH may represent a new target for the design of drugs and therapeutic peptides with desired cell specificity and selectivity.

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Figure 1.
The pH-dependent membrane lysis activity of peptide CL-7 with a transition pH=7.35.

Figure 2.

The cell lysis activity of pH-sensitive lytic peptides (40 μ M) as tested on CHO-K1. Studies were performed in serumfree medium (pH=7.4). No peptide was added in control groups. Cell lysis (LDH release) was normalized to cell death percentage against blank and 100% lysis control. Data represents the mean and SD of at least three independent tests.

Figure 3.

The cell lysis activity of pH-sensitive lytic peptides (40 μ M) as tested on A549 cells. Studies were performed in serum-free medium (pH=7.4). No peptide was added in control groups. Cell lysis (LDH release) was normalized to cell death percentage against blank and 100% lysis control. Data represents the mean and SD of at least three independent tests.

Figure 4.

Cell surface pHs regulated cell lysis activity of pH-sensitive lytic peptide CL-7 and CL-22 as tested on CHO-K1 cells (I), CHO-K1 cells treated with 8-CPT-cAMP (II), and CHO-K1 cells treated with 8-CPT-cAMP, followed by bafilomycin A1 (III). Cell surface pHs corresponding to different treatments were provided in Table 3. Peptide concentrations were fixed at 40 μ M in all experiments. No peptide was added in control groups. Cell lysis (LDH release) was normalized to cell death percentage against both blank and 100% lysis controls. Data represents the mean and SD of at least three independent tests.

Figure 5.

(A) Dynamics of peptide CL-7 induced membrane damages in A549 cells as measured using LIVE/DEAD kit. Fluorescence images were taken after peptide solutions were added at different time points. The percentage of green pixels out of the total green and red pixels was calculated to estimate the cell membrane integrity. (B) Peptide CL-7 binding and insertion induced surface tension changes on lipid monolayer (DPPC/Cholesterol/DPPS=50/10/2.5). Peptide concentration was kept at 10 μ M and the initial surface tension of lipid monolayer was set at 33mN/m.

Figure 6.

(A) The pH affected CL-7 aggregation in solutions (40 μ M) at different pHs. The 1,8-ANS concentration was fixed at 20 μ M and the excitation wavelength was set at 369 nm. Peptide aggregation dissolution was indicated by the red shift and fluorescence intensity decrease of 1,8-ANS emission peak at 500 nm; (B) SEM images of CL-7 aggregates formed in 40 μ M peptide solution at pH=7.4; (C) Measured particle sizes of peptide CL-7 (80 μ M) in solutions after overnight incubation. Data represents the mean and SD of at least three independent tests.

Figure 7.

The pHs affected CD spectrum changes of peptide CL-7 measured in 20 mM NaAc solutions. Data were collected from 250 to 190 nm at an interval of 1.0 nm with an integration time of 2 seconds at each wavelength. Five to ten scans were averaged, smoothed, background subtracted, and converted to mean residue ellipticity (θ) [degrees/(cm² dmol⁻¹)].

Figure 8.

Cytotoxicity (MTT assay) of a pH-sensitive peptide PTP-7b (FLGALFKALSHLL) a normal-cancer cell pair CCD-13Lu and A549 cells. A pH-insensitive peptide PTP-7 (FLGALFKALSKLL) was used as the control. Data represents the mean and SD of at least three independent tests.

Table 1

Cell surface pH and charges measured at physiological pH =7.4

	A549	CCD-13Lu	CHO-K1
Cell surface pH*	7.15±0.17	7.39±0.16	8.40±0.24
Total surface Charges**	1.37±0.08	3.49±0.12	1.23±0.12

* Cell surface pHs were measured using FITC-labelled wheat germ agglutinin.

** Cell surface charges were measured using toluidine blue assay. Charge unit=107 µeq/cell

Table 2

Properties of selected lytic peptides

Peptide	Structure		pH sensitivity [*]	Transition pH ^{**}
	pH=7.4	pH=6.5		
pH-sensitive				
CL-7, FLGALFRALSHLL	α-helix	Coil	2.2	7.35
CL-9, WLGALFHALLSKLL	α-helix	Coil	3.0	6.80
CL-10, WLGALFKALSHLL	α-helix	Coil	2.5	6.60
CL-22, WLGALFKALSHLLGHHPH	α-helix	Coil	13.8	7.15
pH-insensitive ^{***}				
CL-1, FLGALFRALSRL	Coil	Coil	1.2	-

* The ratio calculated from the average IC₅₀ values of a peptide at pH=7.4 and pH=5.5.

** The pHs at which solvated peptides carried zero charges as measured using Nanosizer.

*** A sequence and structure similar but pH-insensitive peptide, CL-1, was used as a control in related studies.

Table 3

8-CPT-cAMP and Bafilomycin induced cell surface pH changes in the medium with physiological pH

		Cell Surface pH.	
		8-CPT-cAMP	Bafilomycin A1
	-		
A549	7.15±0.07	6.55±0.19	8.90±0.12
CHO-K1	8.40±0.24	6.90±0.21	9.06±0.06