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pH-controllable cell-penetrating polypeptide that exhibits cancer targeting

DaeYong Lee, Ilkoo Noh, Jisang Yoo, N. Sanoj Rejinold, Yeu-Chun Kim *

Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Republic of Korea

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ABSTRACT

Helical peptides were naturally-occurring ordered conformations that mediated various biological functions essential for biotechnology. However, it was difficult for natural helical polypeptides to be applied in biomedical fields due to low bioavailability. To avoid these problems, synthetic alpha-helical polypeptides have recently been introduced by further modifying pendants in the side chain. In spite of an attractive biomimetic helical motif, these systems could not be tailored for targeted delivery mainly due to nonspecific binding events. To address these issues, we created a conformation-transformable polypeptide capable of eliciting a pH-activated cell-penetrating property solely at the cancer region. The developed novel polypeptide showed that the bare helical conformation had a function at physiological conditions while the pH-induced helical motif provided an active cell-penetrating characteristic at a tumor extracellular matrix pH. The unusual conformation-transformable system can elicit bioactive properties exclusively at mild acidic pH.

Statement of Significance

We developed pH-controllable cell-penetrating polypeptides (PCCPs) undergoing pH-induced conformational transitions. Unlike natural cell-penetrating peptides, PCCPs was capable of penetrating the plasma membranes dominantly at tumor pH, driven by pH-controlled helicity. The conformation of PCCPs at neutral pH showed low helical propensity because of dominant electrostatic attractions within the side chains. However, the helicity of PCCPs was considerably augmented by the balance of electrostatic interactions, thereby inducing selective cellular penetration. Three polypeptides undergoing different conformational transitions were prepared to verify the selective cellular uptake influenced by their structures. The PCCP undergoing low-to-high helical conformation provided the tumor specificity and enhanced uptake efficiency. pH-induced conformation-transformable polypeptide might provide a novel platform for stimuli-triggered targeting systems.

1. Introduction

α-helical structures mediate protein folding or unfolding, and are the fundamental foundation for the protein assembly and bioactive functions [1,2]. Among various α-helical peptides, cationic helical peptides (CHP) have been used in biomedical fields due to their unique cell-penetrating property. CHPs composed of sterically unhindered and lysine or arginine-rich amino acid residues strongly bind to the lipid plasma membranes via electrostatic attractions, and then distort the arrangement of lipid molecules, thereby being translocated in the cytosol [3,4]. Nevertheless, naturally obtained helical peptides showed the innate limitations such as unsatisfactory packing with polyanions, low delivery effectiveness, and vulnerability to enzymatic degradation and temperature [1,3]. In an effort to be applied biologically, further modification of side chains in the polypeptide effectively enables the elicitation of a bioavailable helical structure that is mainly dependent on three key factors: the length of hydrophobic chain, the bulkiness of side chains, and the surface charge density [1,3,5–7]. The water-affinitive helical polypeptide with proper surface charge density is rendered thermodynamically stable at physiological conditions [1,3,5,8–10]. Based on previous studies, the artificial helical polypeptide (AHP) has successfully mimicked the natural helical conformation, and initiated a new platform for drug and gene delivery systems [1,3,5,8–10]. AHP has been recently utilized as

* Corresponding author.
E-mail address: dohnanyi@kaist.ac.kr (Y.-C. Kim).

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an effective non-viral vector for therapeutic purposes [3,5,8–10]. Generally, AHP, a gene-condensing material, was packed with therapeutic gene and then the complex is passively delivered to the diseased sites, demonstrating the superior therapeutic efficacy in a comparison to the previously reported polycation [3,5,9,10]. The driving force of the high delivery efficiency of AHP is associated with its similar behavior as a natural cell-penetrating peptide, where the helical conformation is allowed to facilitate cell penetration into the membrane by interacting with plasma phospholipids [3,11–14]. However, its delivery system was not well-customized to all the diseases due to the innate limitation of cell-penetrating characteristics, including nonspecific interactions, even with normal cellular plasma membranes [11–14]. The non-specific targeting strategy has caused unexpected side-effects in drug and gene delivery systems [15,16]. Therefore, it is imperative for targeting delivery systems to endow desirable properties only at the diseased region [16]. Comparing normal cells, a specific stimuli-responsive characteristic should be introduced in the delivery systems to possess high selectivity to cancer. In contrast to the normal environments, the tumor extracellular conditions were relatively acidified due to high lactate concentrations, which might be the novel stimulus for selective delivery to cancers [17,18].

Herein, we suggested that pH-controllable cell-penetrating polypeptides (PCCP) regulating pH-adaptable helicity attained cells for specific cancer guidance (Fig. 1). We hypothesized that PCCP was capable of undergoing pH-dependent conformational transition, thereby revealing the cell penetrating characteristic, specifically at the target site (Fig. 1b). PCCP contained low helical propensity at a physiological pH through the electrostatic attractions between carboxylate and protonated amine groups in each side chain (Fig. 1a). Thus, indiscriminate cellular internalization could be expeditiously circumvented by a hiding effect. In contrast, with an acidic environment, the pH-inactivated motif rapidly converted to an intact helical structure whereby the electrostatic interactions between repulsions and attractions were well-balanced throughout the side chains at pH 5–6 (Fig. 1). Below pH 4, the electrostatic repulsions were so dominant that the back bone can be more elongated, bringing about a rapid reduction in helicity (Fig. 1a). Consequently, the conformation of PCCP could change to a helix that possessed a superior cell-penetrating property, but only at cancer sites.

In this study, we synthesized several poly-L-lysine (PLL)-based PCCPs two optimize pH-activated cell-penetrating capability exclusively in cancer cells. The primary amine moieties in PLL provided the further reaction because of the nucleophilicity. Using benzoylation, poly-4-bromobenzoyl-L-lysine (PBL) which was vulnerable to Pd-catalyzed reactions, was successfully synthesized. To determine the protein secondary structure affected by the bulkiness and the additional charge in the side chains, 4-imidazoleacrylic acid and acrylic acid were used as a building block to synthesize poly(4-(2-(imidazole-4-yl)acrylic acid)benzoyl-*co*-4-(4-(imidazole-2-yl)benzoyl-L-lysine) and poly(4-(3-acrylic acid) benzoyl-L-lysine) (PABL).

Based on the synthesis of potential PCCPs. the optimized PCCPs showed two-fold increase of helicity when pH level was reduced to pH 6 (tumor extracellular pH). This design principle was grafted onto cellular conditions to strongly confirm pH-dependent cell penetration. The optimized PCCP exhibited outstanding selectivity only against cancer at low pH, driven by specific cell-penetration.

2. Materials and methods

2.1. PLL synthesis

The PLL synthetic method was utilized as described in previous studies [19–21].

2.2. PBL Synthesis

In a glove box, PLL (1.00 g) was solubilized in anhydrous *N,N′*-dimethylformamide (DMF) (10 mL) with triethylamine (TEA) (2.7 mL, 19.3 mmol), and then 4-bromobenzoyl chloride (3.40 g, 15.48 mmol) was slowly added to the solution. The reaction was allowed to occur at RT for 24 h. A yellowish suspension was precipitated with deionized water used to remove the salt that formed, and washed with water twice. To remove the unreacted agent, the crude product was washed with 1 M NaOH solution three times, and then lypophilized. From this procedure, PBL (2.00 g) was obtained.

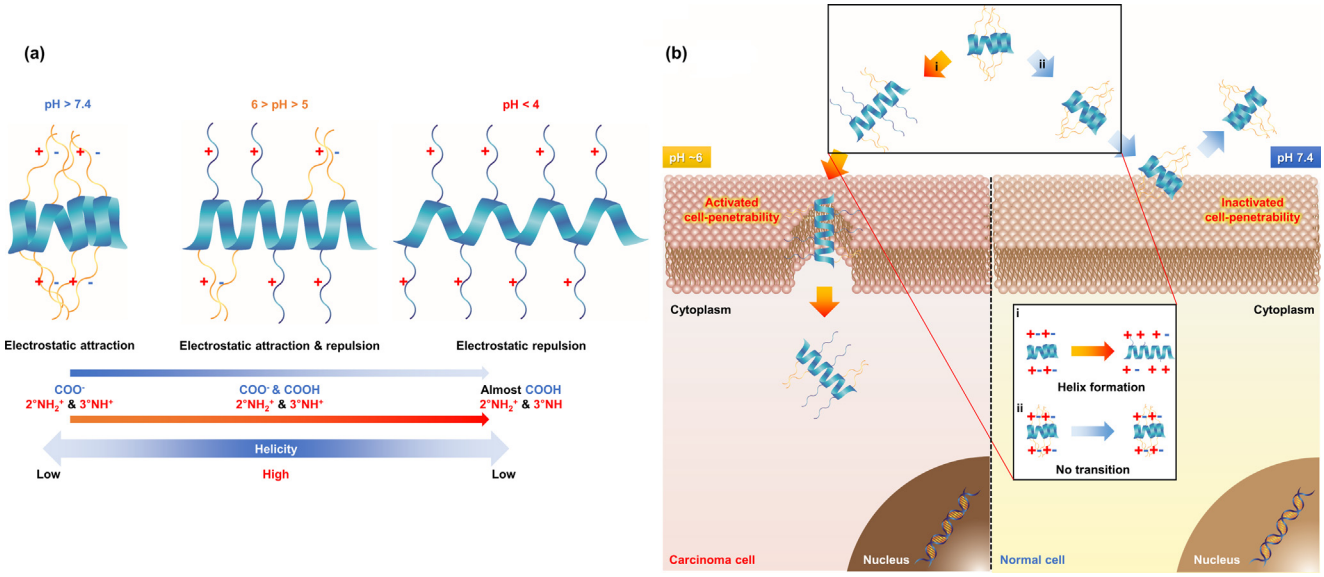

Fig. 1. Design of PCCPs, and proposed mechanism of pH-controllable helicity and selective cellular penetration. (a) Proposed mechanism of PCCP possessing a pH-activated cell penetrating property exclusively at the tumor extracellular matrix. 2° and 3° indicate "secondary" and "tertiary", respectively. (b) Schematic illustration of the PCCP undergoing pH-dependent conformational transition induced by the charge balances of two opposite ions.