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Inter-polyelectrolyte nano-assembly induces folding and activation of functional peptides

Naohiko Shimada, Hirotaka Kinoshita, Shuichi Tokunaga, Takuma Umegae, Nozomi Kume, Wakako Sakamoto, Atsushi Maruyama *

Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 B-57, Nagatsuta, Midori, Yokohama 226-8501, Japan

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ABSTRACT

Insufficient solubility, fragile folding structure and short half-life frequently hamper use of peptides as biological reagents or therapies. To enhance the peptide function, the effect of complexation of the peptides with ionic graft copolymers with water-soluble graft chains was tested in this study. Amphiphilic anionic peptide E5 acquires membrane disrupting activity at acidic pH due to folding from the random coil state to an ordered α-helical structure. Aggregation and imprecise folding of the peptide limited membrane disrupting activity of the peptide. In the presence of a cationic graft copolymer, E5 and its analogs adopted an ordered conformation without aggregation. The mixture of the peptides and the copolymer functioned more efficiently than peptide alone at not only acidic pH but also neutral pH at which the peptide alone had no activity. Similarly, a cationic peptide was successfully folded and activated by an anionic graft copolymer. Thus, our analysis indicated that spontaneous nano-assembly of ionic peptides with graft copolymers having opposite ionic charges triggers the folding of peptides without loss of solubility, leading to enhanced bioactivity.

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1. Introduction

Polyelectrolytes spontaneously form inter-polyelectrolyte complexes (IPECs), also known as polyion complexes (PICs), with oppositely charged polyelectrolytes [1–3]. Due to their thermodynamic stabilities and/or stoichiometric properties, IPECs have been applied to protein separation [4,5], colloid stabilization [6,7], and membrane preparation [8,9]. A variety of micro- or nano-structures such as porous microspheres [10], nanocapsules [11], and well-organized films [12] have been prepared from IPECs. Upon IPEC formation, the conformation of the polyelectrolyte frequently changes from an extended coil structure to a highly condensed and compacted globule structures (i.e., the polyelectrolyte undergoes the coil-to-globule transition) [1]. Negatively charged (anionic) DNAs also form IPECs with positively charged polyelectrolytes, or polycations, resulting in condensed and compacted conformation of the DNA [13]. The resulting IPECs often aggregate and precipitate out of aqueous media. Assembly of IPECs can be controlled by employing block copolymers consisting of a polyelectrolyte segment and a hydrophilic polymer segment. Core-shell type polyion complex micelles assembled with condensed DNA IPECs in the core are promising gene delivery carriers in vitro and in vivo [14–17]. Well-organized polymer micelles [18] and bilayer vesicles [19] have also been constructed from IPECs based on oppositely charged block copolymers.

Our group has long-standing interest in the IPEC formation between DNA and graft copolymers composed of a polycation main chain and abundant water-soluble graft chains (Fig. 1A). The graft copolymers composed of more than 80 wt.% graft chains and less than 20 wt.% positively charged (cationic) main chain form water-soluble IPECs (wsIPECs) with DNA [20]. We showed that the copolymer facilitates folding and assembling DNA to ordered structures. In the wsIPEC, DNA duplex and triplex conformations were thermodynamically stabilized [21–24]. Structural transitions of DNA from right-handed B-type to left-handed Z-type can also be controlled by the copolymers [25,26]. Kinetically, duplex, triplex, and quadruplex DNA formation were accelerated by 2–3 orders of magnitude in the presence of IPECs [27–29]. Conversion to the most stable structure from metastable structures of DNA was promoted in IPECs, suggesting that IPECs have nucleic acid chaperone-like function that facilitates folding and assembly of nucleic acid structures [27,30–34]. Molecular chaperon is a class of cellular proteins whose function is to ensure that the folding and assembly of biomolecules occur correctly. They do not form part of the final structure nor do they necessarily possess steric information specifying assembly [35]. Once the final structure has been formed, they spontaneously dissociate from the target molecules. Though our cationic copolymers facilitate assembly of nucleic acids, the copolymers do not dissociate from the target molecules after the assembly and in some case the copolymer influences the final structure. Hence, we describe the activity of the copolymers as “chaperone-

* Corresponding author.
E-mail address: amaruyama@bio.titech.ac.jp (A. Maruyama).

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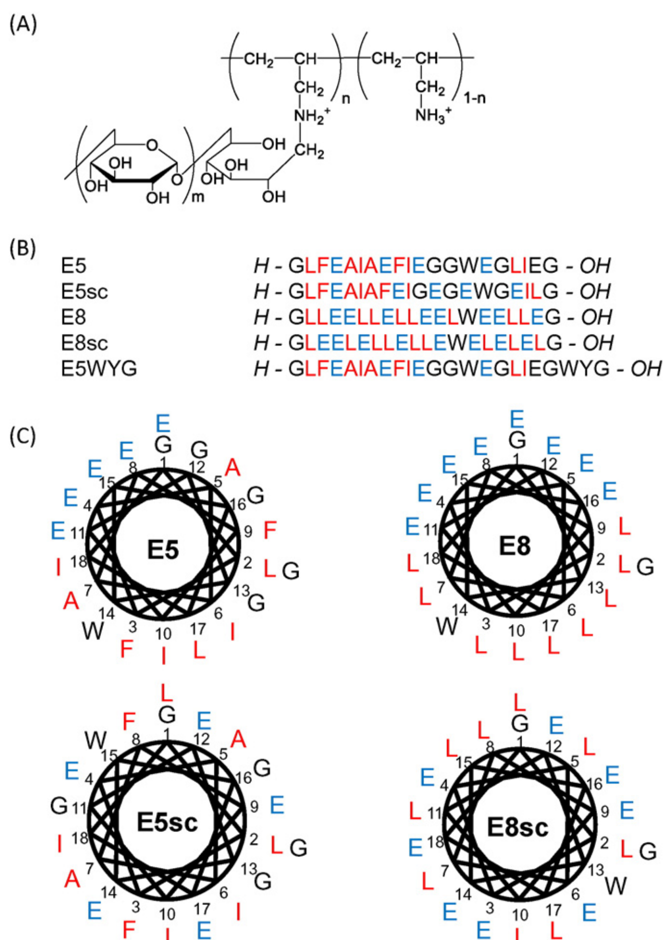

Fig. 1. (A) Structural formula of PAA-g-Dex. (B) Sequences of peptides used in this study. (C) Helical wheel representations of E5 and analogues.

like" activity. Shielding of the electrostatic potential along anionic DNA strands by the cationic copolymer appears to be responsible for these thermodynamic and kinetic observations [24,28].

Peptides are recognized for being highly selective, effective and safety. Approximately 140 peptide therapeutics are currently in clinical trial [36]. Peptides are often not directly suitable for biotechnological and medicinal uses because they have intrinsic weaknesses, including poor chemical and physical stability, fragile folding structure, poor solubility (tendency to aggregate) and short half-life (fast elimination) [36].

We hypothesized that folding, solubility, and function of ionically charged peptides could be manipulated through wsIPEC formation with the graft copolymer having opposite ionic charges. Here we tested ionic graft copolymers to fold and activate ionic peptides through interpolyelectrolyte nano-assembly.

2. Experimental section

2.1. Materials

Peptides (purity >98%) used in this study (Fig. 1B) were purchased from Genenet. Poly(allylamine) hydrochloride (PAA·HCl), kindly supplied by Nitto Boseki Co. in aqueous solution, was purified by precipitation from methanol. The cationic graft copolymer, PAA-*g*-Dex (Fig. 1A), was prepared by reductive amination of PAA·HCl (M_w: 5×10^3) and dextran (M_w: 7×10^3) as we previously reported [21]. The composition of the resulting copolymer was confirmed by ^{1}H-NMR in D_2O to be 92 wt.% dextran and 8 wt.% PA. β-galactosidase (β-Gal) and fluorescein di-β-D-galactopyranoside (FDG) were purchased from Funakoshi and AnaSpec, respectively. 1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) and *N*-(carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (PEG2K-DSPE) were purchased from NOF Corporation. Other reagents were purchased from Wako Pure Chemical Industries.

2.2. Circular dichroism measurements

Circular dichroism (CD) spectra were measured on a Jasco J-820 CD spectropolarimeter equipped with a Peltier temperature controller. Samples were placed in 10-mm quartz cells, and each spectrum was collected from 200 to 250 nm at a scan rate of 100 nm/min with a response time of 2 s. Data were averaged over four accumulations. The concentrations of peptides in 0.5 mM NaOH were determined by UV spectroscopy (Shimadzu UV-1650PC) using a molar extinction coefficient of 5500 $M^{-1} \times cm^{-1}$ (expressed as a tryptophan) at 280 nm. CD spectra of peptides (10 μM) in the absence of PAA-g-Dex were measured at various pH values. Solution pH was adjusted using 0.5 mM NaOH or 100 mM HCl; pH was determined at 25 °C. CD spectra of peptides in the presence of PAA-*g*-Dex were measured in water at pH 7 (adjusted using 0.5 mM NaOH or 100 mM HCl) or 10 mM Tris–HCl (pH 7) containing 140 mM NaCl at 25 °C.

The equilibrium dissociation constants (K_d) of cationic copolymer (PAA-*g*-Dex) and peptides were defined as follows:

$$K_d = \frac{[CP][Pep]}{[CP\text{-}Pep]} \tag{1}$$

where [*CP*], [*Pep*], and [*CP-Pep*] are concentrations of free cationic copolymer, free peptide, and the copolymer/peptide complex, respectively. The observed mean molar residue ellipticity at 222 nm (θ_{obs}) is given by:

$$\theta_{obs} = \theta_{min}\frac{[Pep]}{[Pep]_0} + \theta_{max}\frac{[CP\text{-}Pep]}{[Pep]_0} \tag{2}$$

where $[Pep]_0$ is total concentration of peptide, and θ_{min} and θ_{max} are polarities of free peptide and bound peptide, respectively. Combining Eq. (1) and (2), yields the expression for θ_{obs}:

$$\theta_{obs} = \left[(\theta_{max} - \theta_{min})\frac{([CP]_0 + [Pep]_0 + K_d) - \sqrt{([CP]_0 + [Pep]_0 + K_d)^2 - 4[CP]_0[Pep]_0}}{2[Pep]_0}\right] + \theta_{min} \tag{3}$$

In this equation, $[CP]_0$ is total concentration of cationic copolymer. Each K_d value was determined by fitting of the experimental curve to Eq. (3).

2.3. Measurement of leakage from liposomes induced by peptides complexed with PAA-g-Dex

A dry lipid film composed of DOPC/PEG2K-DSPE (499:1) was prehydrated with water at 37 °C for 3 min. Liposomes were prepared by hydration of the lipid film with 200 μL of 10 mM HEPES-NaOH (pH 7.4) containing 40 mM NaCl, 100 mM sucrose, and 1 mM FDG at 37 °C for 2 h. After hydration, the solution was diluted with 1 mL of Buffer A (10 mM HEPES-NaOH, pH 7.4, containing 140 mM NaCl). Liposomes were collected by centrifugation (5000 *g*) at 4 °C for 10 min. The pellet was washed three times with Buffer A, and liposomes (final lipid concentration 24 μM) were suspended in Buffer A containing 81.6 μg/mL β-Gal or in a solution of 10 mM MES-NaOH (pH 5.4) containing 140 mM NaCl and 408 μg/mL β-Gal. The fluorescence intensities (λ_{ex} 490 nm and λ_{em} 520 nm) of samples of the suspension in the presence of peptides (5 μM) and/or PAA-g-Dex (5 μM) were recorded by FP-6500 spectrofluorometer at 25 °C as a function of time.

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