pH-Dependent Dissociation of Carbohydrate Ligand Polycations from DNA Ternary Complexes

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Here, we have demonstrated that carbohydrate ligand polycations are pH-dependently dissociated from DNA polyion complexes. In response to pH, DNA ternary complexes have dissociated the ligand polycations, resulting in DNA binary complexes. Namely, the ternary complexes of DNA, a poly(L-histidine) modified with aminoethyl groups, PLH-NH_2, and a poly(L-lysine) conjugated with lactose molecules, PLL-Lac, at pH 7.5 have dissociated the PLL-Lac polycations by the protonation of imidazole groups of PLH-NH_2 at pH 6.0. The pH-dependent dissociation of the ligand polycations from DNA polyion complexes at endosomal pH is expected to offer unique design for DNA delivery systems.

In gene delivery systems, the formation of DNA–carrier polyion complexes is a key factor for new design of efficient delivery.[1] The carrier design typically focuses on the ability of a ligand to bind tightly to its target. The ligand–receptor complexes on the cell plasma membrane are internalized into acidic endosomal vesicles where they are subjected to a significant pH change from pH 7 to 5.[2] Subsequently, the targeting ligands are routed to the degradation in the acidic compartment following endosomal vesicles or are recycled to the cell surface.[3] Such fate of ligands leads us to create the DNA polyion complexes dissociating ligand molecules in response to pH. In general, targeting ligands are unnecessary to make DNA complexes escape from endosomal vesicles. The endosomal escape is one of the critical factors for efficient gene delivery. If the targeting ligands are conjugated with pH-sensitive polycations, i.e., formation of binary complexes, the pH-dependent peculiar property of the polycations is considered to diminish. The formation of the ternary complexes between ligand polycations, pH-sensitive polycations, and DNA is expected to preserve the property of the pH-sensitive polycations. Here, we communicate that ligand polycations are pH-dependently dissociated from DNA polyion complexes. The concept that DNA ternary complexes dissociate ligand polycations in response to endosomal pH, resulting in DNA binary complexes, has no precedent, to the best of our knowledge.

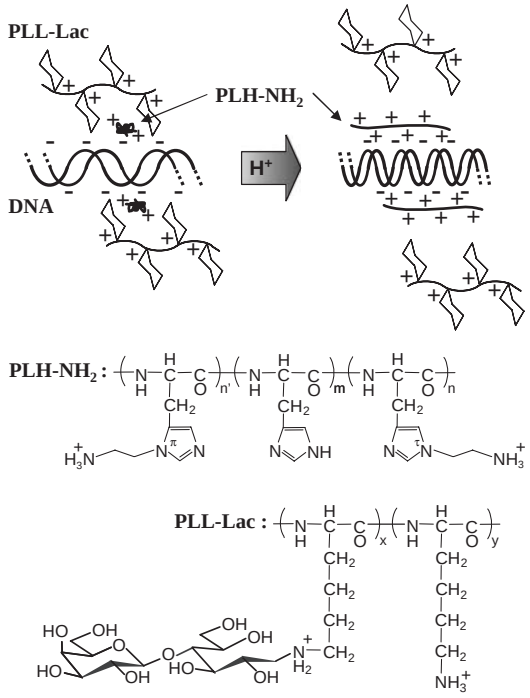

Figure 1. Experimental schema of the pH-dependent dissociation of ligand polycations from DNA polyion complexes. PLH-NH_2; aminated poly(L-histidine). PLL-Lac; lactosylated poly(L-lysine).

The experimental schema of the pH-dependent dissociation of carbohydrate ligand polycations from DNA polyion complexes is depicted in Figure 1. We have chosen a poly(L-lysine) conjugated with lactose (4-*O*-β-D-galactopyranosyl-D-glucose), PLL-Lac, as carbohydrate ligands for an asialoglycoprotein re-

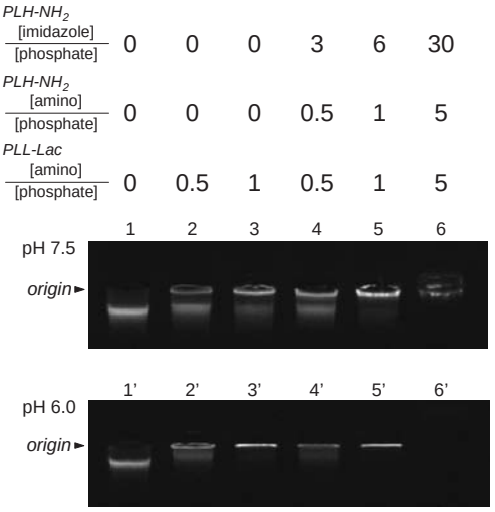

Figure 2. Analysis of the pH-dependent formation of the DNA/PLH-NH_2/PLL-Lac ternary complex by agarose gel electrophoresis in the presence of EtBr. DNA/PLH-NH_2/PLL-Lac mixtures were loaded at pH 7.5 (lanes 1–6) or pH 6.0 (lanes 1′–6′). Lanes 1 and 1′, DNA alone. Lanes 2, 2′, 3, and 3′, DNA/PLL-Lac mixtures at different ratios relative to amino groups per phosphate group of DNA ([amino]/[phosphate]). Lanes 2 and 2′, 0.5×; lanes 3 and 3′, 1×. Lanes 4–6 and 4′–6′, DNA/PLH-NH_2/PLL-Lac mixtures at different respective ratios ([amino]/[phosphate]). Lanes 4 and 4′, 0.5×/0.5×; lanes 5 and 5′, 1×/1×; lanes 6 and 6′, 5×/5×. Corresponding ratios relative to imidazole groups per phosphate group are also indicated ([imidazole]/[phosphate]). Lanes 4 and 4′, 3×; lanes 5 and 5′, 6×; lanes 6 and 6′, 30×.

ceptor on hepatocytes.[4] The conjugation was carried out by the reductive amination[5] between the reducing end of lactose and ε-amino groups of poly(L-lysine) (P_n: 40), resulting in 80 mol % amino group modification. As accompanied polycation, a poly(L-histidine) modified with aminoethyl groups, i.e., aminated poly(L-histidine),[6] PLH-NH_2 (P_n: 60), has been chosen. The PLH-NH_2 has many imidazole groups with a pK_a around 6.0 as well as aminoethyl groups with a pK_a around 10. The content of aminoethyl histidine was 15 mol %.

Accordingly, the pH-dependent dissociation of PLL-Lac from DNA polyion complexes was examined by agarose gel electrophoresis. In Figure 2, DNA was stained by ethidium bromide (EtBr), which was intercalated between base pairs of duplex DNA. The intercalation of EtBr increases the fluorescence, so that EtBr is effective for the detection of DNA. Each lane contains the same amount of DNA, and lanes 2–6 (and lanes 2′–6′) contain increasing amounts of polycation (PLL-Lac and/or PLH-NH_2).[7] At pH 7.5, the band retained at the origin increased as amino/phosphate ratio increased. Therefore, the formation of the DNA polyion complex with polycations was confirmed. It should be noted that the band retained at the origin at pH 7.5 (lane 6) disappeared at pH 6.0 (lane 6′) in the higher mixing ratios ($[\text{amino group}]_{\text{PLL-Lac}}/[\text{phosphate group}]_{\text{DNA}} = 5$; $[\text{amino group}]_{\text{PLH-NH2}}/[\text{phosphate group}]_{\text{DNA}} = 5$; $[\text{imidazole group}]_{\text{PLH-NH2}}/[\text{phosphate group}]_{\text{DNA}} = 30$). These results suggest that the protonation of imidazole groups of PLH-NH_2 at pH 6.0 induced the coil–globule transition of DNA and inhibited the EtBr intercalation.[8]

In above experiments at lane 6, however, the formation of the polyion complex with PLL-Lac was unclear. Therefore, we labeled PLL-Lac with fluorescein isothiocyanate (FITC)[9] and examined whether FITC-labeled PLL-Lac formed the polyion complex with DNA by agarose gel electrophoresis in the absence of EtBr, as shown in Figure 3. Other experimental conditions are the same as Figure 2. At pH 7.5 in the higher mixing ratios, the band was also retained at the origin (lane 6). It is, therefore, confirmed that the PLL-Lac formed the polyion complex with DNA at pH 7.5; namely, DNA, PLH-NH_2, and PLL-Lac formed the ternary complexes at pH 7.5. At pH 6.0, on the other hand, it is worth noting that the FITC-labeled PLL-Lac migrated into the minus pole of the gel (lane 6′). The migration into the minus pole suggests that the PLL-Lac polycation was dissociated from the DNA complex.

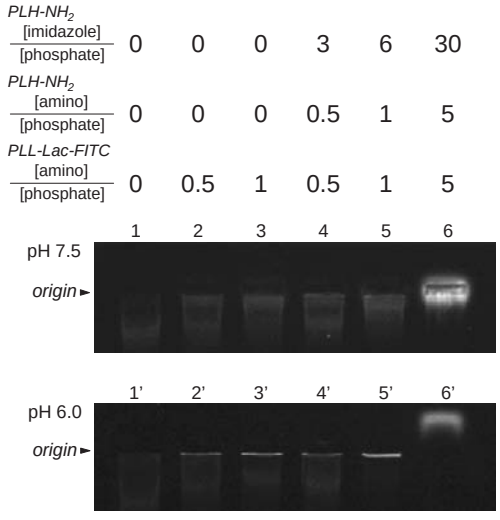

Figure 3. Analysis of the pH-dependent formation of the DNA/PLH-NH_2/PLL-Lac ternary complex by agarose gel electrophoresis in the absence of EtBr, where PLL-Lac was labeled with FITC. Then, the mixtures of DNA, PLH-NH_2, and FITC-labeled PLL-Lac (PLL-Lac-FITC) were loaded at pH 7.5 (lanes 1–6) or pH 6.0 (lanes 1′–6′). Other experimental conditions are the same as Figure 2.

Taking these results into account, the ternary complexes of PLL-Lac, PLH-NH_2, and DNA at pH 7.5 have dissociated the PLL-Lac polycations by the protonation of imidazole groups of PLH-NH_2 at pH 6.0. The carbohydrate lactose molecules conjugated to the polycation poly(L-lysine) can work as solubilizer[10] of the DNA polyion complexes and weaken the electrostatic interaction between the phosphate groups of DNA and the amino groups of poly(L-lysine). The protonated imidazole groups of PLH-NH_2 are considered to have taken the phosphate groups of DNA from the amino groups of PLL-Lac. The resulting binary complexes between PLH-NH_2 and DNA exhibited the nonlinear change of the solution properties, such as acid–base titration and turbidity, at pH 6.0 (results not shown). The transition pH value (pH 6.0) can be shifted, if necessary, by introducing hydrophilic or hydrophobic groups[11] to the PLH-NH_2 or by replacing poly(L-histidine) with other pH-sensitive polymers.[12] The concept of the pH-dependent dissociation of ligand polycations from DNA polyion complexes at endosomal pH (pH 6) in this study is expected to offer unique design for DNA delivery systems.

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References and Notes

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7 Experimental procedure is as follows: Salmon testes DNA sodium salt was dissolved in phosphate buffered saline (pH 7.4) at 1 mg/mL. The DNA solution was added to the aqueous solutions of the polypeptide in 50 mM sodium phosphate buffer (pH 7.5 or pH 6.0) at various polypeptide/DNA ratios. The final concentration of DNA was adjusted to 60 μg/mL. After 2 h of incubation at room temperature, 10 μL of the samples (corresponding to 0.6 μg of DNA) were electrophoresed (50 V and 15 min) through a 1% agarose gel containing 1 μg/mL of ethidium bromide using 50 mM sodium phosphate buffer (pH 7.5 of pH 6.0).
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9 Labeling procedure is as follows: PLL-Lac (10 mg) and FITC (0.41 mg) were dissolved in the mixed solvent of dimethyl sulfoxide (1 mL) and distilled water (0.7 mL) containing 20 μL of triethylamine. The reaction mixture was incubated at 40 °C for 6 h, followed by thorough ultrafiltration to remove unreacted FITC. The resulting polypeptide was obtained by freeze-drying.
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