Construction of Artificial Viral Capsids Encapsulating Short DNAs via Disulfide Bonds and Controlled Release of DNAs by Reduction

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To construct an artificial viral capsid encapsulated short single-stranded DNA, a *β*-annulus peptide conjugated with ssDNA through a disulfide bond at the N-terminus (DNA-SS-*β*-Annulus) was synthesized. The DNA-SS-*β*-Annulus conjugate self-assembled into spherical structures ranging in the size of 36–60 nm. ssDNA was released from the capsids via the reduction of disulfide bonds.

Keywords: Artificial viral capsid, Nanocarrier, DNA, Disulfide bond

Spherical viral capsids are natural supramolecular nanocapsules with icosahedral symmetry, which are formed via self-assembly from capsid proteins. They have attracted significant attention as scaffolding materials, which can act as drug carriers, vaccine platforms, and nanoreactors because of their specific properties, such as discrete nanospace, good cell-transfection ability, and biodegradability.[1] Particularly, the encapsulation of nucleic acids in the discrete nanospace of spherical viral capsids has made the improved resistance of nucleic acids in cells against enzymatic degradation possible, which can be applied as a nanocarrier for the delivery of nucleic acid drugs.[1c, 2]

In biological systems, disulfide bonds comprising cysteines play an important role in the stability of the three-dimensional structures of proteins, and in the control of certain protein functions[3], which are susceptible to the redox environment in the cytoplasm. The reductive cleavage of disulfide bonds is utilized to release drugs in the cytoplasm from nanomaterials, such as polymeric micelles.[4] However, to the best of our knowledge there are fewer reports on the controlled-release of guest molecules by reductive cleavage of disulfide bonds when viral capsids are used as scaffold materials.[5]

We have previously demonstrated that a synthetic 24-mer *β*-annulus peptide fragment (INHVGGTGGA IMAPVAVTRQLVGS), which is a structural motif of the internal skeleton of the tomato bushy stunt virus, self-assembled into hollow nanocapsules (artificial viral capsid) ranging in the size of 30–50 nm in water.[6, 7] The pH-dependent zeta-potentials of the artificial viral capsid indicate that the C-terminus of the peptide is directed toward the exterior surface, while the N-terminus is directed toward the interior.[8] By utilizing this feature, we have reported that the C-terminus modification allowed the decoration of the exterior surface of the artificial viral capsid with gold nanoparticles,[9a] coiled-coil spikes,[9b] and ssDNAs.[9c] Moreover, we have demonstrated the encapsulation of anionic dyes, a long DNA (M13 phage DNA),[8] and the preparation of quantum dots (CdTe)[10a] via electrostatic interaction in the cationic interior of the artificial viral capsid. Furthermore, by employing the proper N-terminus modification, fluorescent ZnO nanoparticles[10b] and His-tagged GFP[10c] have been encapsulated in the artificial viral capsid.

Here, we report the construction of an artificial viral capsid encapsulating single-stranded DNA (ssDNA) via the self-assembly of the *β*-annulus peptide modified with ssDNA at the N-terminal through a disulfide bond, wherein the ssDNA was directed to the interior (Figure 1). Linking of *β*-annulus peptide with DNA via disulfide bond ensures the encapsulation of short DNAs, which has been difficult to achieve so far. The controlled-release of the ssDNA from the capsid can be achieved by reductive cleavage of disulfide bonds under intracellular reducing environment.

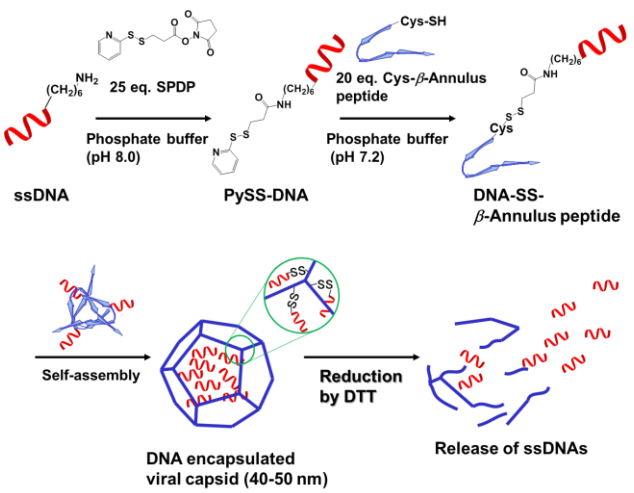
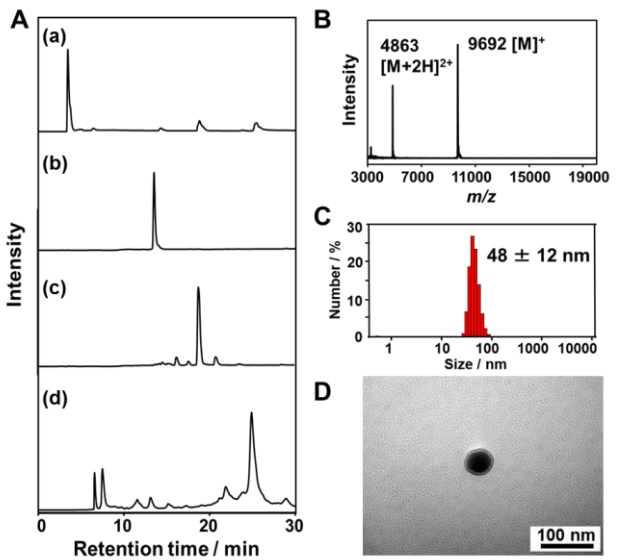


Figure 1. Schematic illustration of the ssDNA encapsulated in an artificial viral capsid through a disulfide bond, and the release of ssDNA by reduction using DTT.

A 23-mer ssDNA (TCTACAAAGGGAAGCCC TTTCTG) bearing an amino group via a hexamethylene chain at the 5′ end was reacted with 3-(2-pyridyldithio) propionic acid *N*-hydroxysuccinimide ester (SPDP) to obtain a ssDNA bearing pyridyldisulfide group at the 5′ end (PySS-DNA). A *β*-annulus peptide containing Cys at the N-terminus (CINHVGGTGGAIMAPVAVTRQLVGS: Cys-*β*-Annulus) was synthesized using a standard Fmoc-protected

solid-phase method. A subsequent disulfide-exchange reaction of PySS-DNA with the excess amount of thiol at the N-terminus of the Cys-*β*-Annulus afforded the *β*-annulus peptide to be modified with the ssDNA via a disulfide bond (DNA-SS-*β*-Annulus). In the reversed-phase HPLC chart, the reaction mixture displayed one main peak at 24.9 min, which was different from the retention time of SPDP, ssDNA, and PySS-DNA (Figure 2A). The purified product was confirmed to be DNA-SS-*β*-Annulus using MALDI-TOF-MS (m/z = 9692 $[M]^+$) (Figure 2B). The dynamic light scattering (DLS) of 50 μM DNA-SS-*β*-Annulus in sodium phosphate buffer (pH 7.1) exhibited the formation of 48 ± 12-nm assemblies (Figure 2C). The transmission electron microscopy (TEM) image of the aqueous solution of DNA-SS-*β*-Annulus also exhibited the formation of spherical assemblies of approximately 50 nm in diameter (Figure 2D). The structure of the assembly of DNA-SS-*β*-Annulus is comparable to that of the unmodified artificial viral capsid,[6] indicating that the modification of



ssDNAs at the N-terminus of the *β*-annulus peptide minimally affect the capsid structure.

Figure 2. (A) Reversed-phase HPLC chart of (a) SPDP, (b) ssDNA, and (c) PySS-DNA detected at 260 nm, eluted with a linear gradient of CH_3CN / 0.1 M NH_4HCO_2 aq (0 / 100 to 100 / 0 over 95 min). (d) DNA-SS-*β*-Annulus detected at 260 nm, eluted with a linear gradient of CH_3CN / 0.1 M NH_4HCO_2 aq (10 / 90 to 100 / 0 over 95 min). (B) MALDI-TOF-MS of the purified DNA-SS-*β*-Annulus (matrix: 3-HPA). (C) Size distributions obtained from DLS analysis for 50 μM DNA-SS-*β*-Annulus in 10 mM sodium phosphate buffer (pH 7.1). (D) TEM image of 50 μM DNA-SS-*β*-Annulus in 10 mM sodium phosphate buffer stained with 2% phosphotungstic acid.

We have previously reported that artificial viral capsids decorated with ssDNAs on the exterior surface were self-assembled from *β*-annulus peptides modified with ssDNA (dA_{20} and dT_{20}).[9c] The ssDNA-modified peptides formed spherical assemblies with diameters of 46–150 nm, although aggregations were observed at higher concentrations (>50 μM). Conversely, the concentration dependence of the size distribution in DLS measurement indicated that DNA-SS-*β*-Annulus formed assemblies with sizes ranging from 30 to 50 nm at a concentration of 25–100 μM (Figure 3A). Since highly charged DNA can be condensed by multivalent counterions,[11] it is likely that the artificial viral capsid encapsulated ssDNA is stabilized by electrostatic condensation between the ssDNA and the cationic interior of the capsid.

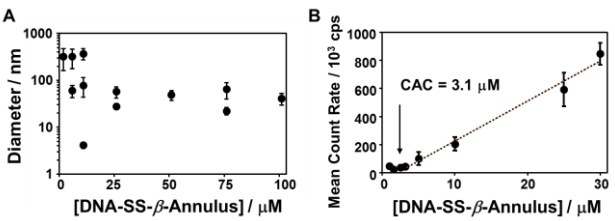


Figure 3. Concentration dependence of size distribution (A) and scattering intensity (B) obtained from DLS for the aqueous solution of DNA-SS-*β*-Annulus in 10 mM sodium phosphate buffer (pH 7.1) at 25°C.

The concentration dependence of the scattering intensity of the aqueous solution of DNA-SS-*β*-Annulus revealed that the critical aggregation concentration (CAC) of DNA-SS-*β*-Annulus was 3.1 μM (Figure 3B). DLS analysis and TEM images of DNA-SS-*β*-Annulus in water at various pH values indicated that the peptide could stably self-assemble in the pH range of 4–11, whereas the peptide aggregated at pH 2 (Figure S1).

To confirm the controlled-release of the encapsulated ssDNA by reductive cleavage of disulfide bonds, the artificial viral capsid was reacted with 10 mM dithiothreitol (DTT) for 2 h in 10 mM sodium phosphate buffer (pH 7.1). A new peak appeared in the reversed-phase HPLC chart after the reaction with DTT, and the peak derived from the DNA-SS-*β*-Annulus disappeared (Figure 4A). The MALDI-TOF-MS of the reaction mixture revealed the existence of Cys-*β*-Annulus (m/z = 2409) and ssDNA bearing a thiol group (m/z =7291) (Figure 4B). DLS analysis and TEM images of the reaction mixture revealed that the spherical structures were disrupted (Figure 4C and 4D). The unstructured aggregates observed on TEM images (Figure 4D) were also observed in the aqueous solution of Cys-*β*-Annulus without ssDNA (Figure S2). These results indicated that the ssDNA encapsulated in the artificial viral capsid was successfully released by reductive cleavage of disulfide bonds, which was accompanied by the destruction of the capsid. It is probable that the destruction of the capsid might be caused by cancellation of electrostatic condensation between the ssDNA and the cationic interior of the capsid.

We also attempted to release ssDNAs from artificial viral capsids using 10 mM reduced glutathione (GSH), which is the most abundant substance in cells, that act as a reducing agent in 10 mM sodium phosphate buffer (pH 7.1) (Figure S3). The peak area derived from the DNA-SS-*β*-Annulus decreased to 63% after a 2-h reaction with GSH, and to 11% after 18 h of reaction in the reversed-phase HPLC chart (Figure S3). The MALDI-TOF-MS of the reaction mixture showed the existence of substances produced by the disulfide-exchange reaction (Figure S3).

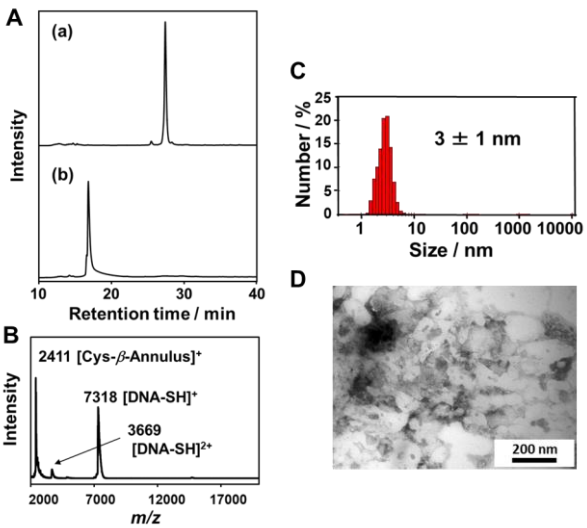


Figure 4. The reduction of DNA-SS-*β*-Annulus ([DNA-SS-*β*-Annulus] = 50 μM, [DTT] = 10 mM) in 10 mM sodium phosphate buffer (pH 7.1) at 25°C. (A) Reversed-phase HPLC chart of DNA-SS-*β*-Annulus (a) before addition of DTT and (b) 2 h after addition of DTT detected at 260 nm, eluted with a linear gradient of CH_3CN / 0.1 M NH_4HCO_2 aq (100 / 0 to 0 / 100 over 70 min), (B) MALDI-TOF-MS (matrix: 3-HPA), (C) DLS, and (D) TEM image for the solution of 50 μM DNA-SS-*β*-Annulus 2 h after the addition of 10 mM DTT.

DLS analysis and TEM images of the mixture showed the co-existence of intact artificial viral capsids and collapsed aggregates (Figure S3). Therefore, ssDNAs may have been partially released due to incomplete reduction using 10 mM GSH. The slower reduction rate of GSH compared to that of DTT might be caused by smaller redox potential of GSH (-0.24 V) than that of DTT (-0.33 V).

In conclusion, the results of this study demonstrated that artificial viral capsid encapsulated ssDNA was self-assembled from *β*-annulus peptide modified with ssDNA at the N-terminus through a disulfide bond. Further, it was observed that the reductive cleavage of the disulfide bond of the artificial viral capsid by DTT or GSH caused the controlled-release of ssDNA with the destruction of the capsid. We envision that this system can be applied to drug delivery systems for short chain nucleic acid drugs, such as siRNA and antisense DNA. When the reduction condition is properly selected, it will be possible to release DNAs without the destruction of capsid. If it is possible, further applications can be developed such as reversible modification of thiol-modified materials in artificial viral capsid.

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Graphical Abstract

Textual Information

A brief abstract (required)	We synthesized a *β*-annulus peptide (DNA-SS-*β*-Annulus) modified with short single-stranded DNA via a disulfide bond at the N-terminus to construct an artificial viral capsid encapsulating ssDNAs. The DNA-SS-*β*-Annulus conjugate self-assembled into a spherical structure with the size of 36 - 60 nm. The viral capsids caused the release of ssDNAs via reduction of the disulfide bonds.
Title(required)	Construction of Artificial Viral Capsids Encapsulating Short DNAs via Disulfide Bonds by Self-assembly
Authors' Names(required)	Yoko Nakamura, Hiroshi Inaba, and Kazunori Matsuura

Graphical Information