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pH-induced fabrication of DNA/chitosan/ α -ZrP nanocomposite and DNA release

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Abstract

With positively charged chitosan as an intermediary, herring sperm DNA was intercalated into the interlayer galleries of negatively charged α -ZrP to form DNA/chitosan/ α -ZrP ternary hybrids at pH 5.5. Fourier-transform IR, x-ray diffraction and scanning electron microscopy confirmed not only the coexistence of DNA, chitosan and α -ZrP in the composite but also the layered composite structure with an interlayer distance of 4.25 nm. Circular dichroism (CD) and UV spectroscopic studies disclosed that the restraint of DNA by the layered α -ZrP favors stabilization of the double-helical conformation of DNA and enhances the denaturation temperature. The intercalated DNA can be effectively released from the ternary nanocomposites at pHs higher than 6.5, and the released DNA displayed a similar CD spectrum to that of free DNA. The current research displays the promising potential to obtain a non-viral gene vector by intercalating DNA into negatively charged inorganic layered materials in the presence of a positively charged intermediary.

 Online supplementary data available from stacks.iop.org/Nano/21/105102/mmedia

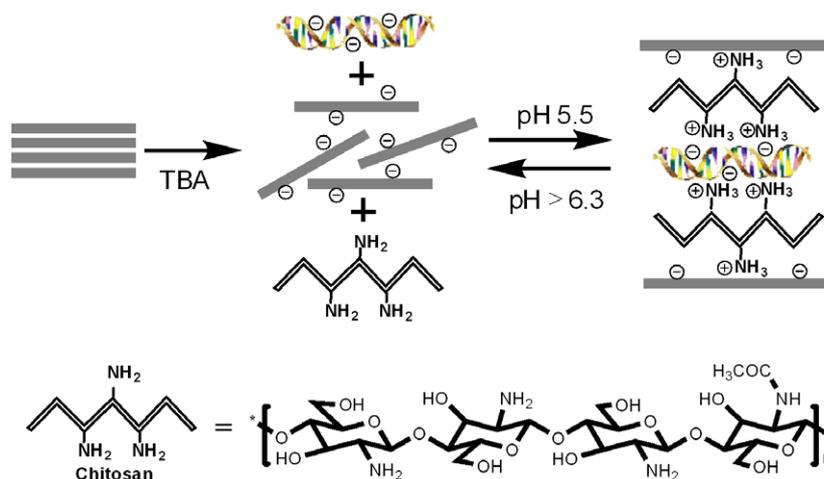
(Some figures in this article are in colour only in the electronic version)

1. Introduction

Bioinorganic nanocomposites, combining biomolecules and inorganic materials at the nanoscale, have attracted considerable attention due to their potential applications as biomedical materials, biosensors, membrane materials for food processing and water purification [1, 2]. In fact, many bioinorganic nanocomposites with special functions, such as hydroxyapatite/collagen composite as the reinforcing material for bone repairing formed by simultaneous titration coprecipitation, the promising platform for biosensor construction formed by encapsulating enzymes into aqueous sol-gel matrices and a clay composite for drug delivery by

intercalating 5-fluorouracil into montmorillonite, have been reported [3–5]. Recently, bio-nanocomposites formed by DNA and inorganic nanomaterials have been reported as non-viral vectors for gene therapy [6–9]. To avoid the nuclease-induced degradation of DNA immobilized on the surface of inorganic nanoparticles [10, 11], great efforts have been devoted to preparing bio-nanocomposites encapsulating DNA using layered inorganic materials for their regular layered structure and expandable interlayer space. In fact, DNA can be easily intercalated into positively charged layered materials via ion-exchange, coprecipitation or delamination assembly due to the low isoelectric point of DNA (pI 4.0–4.5). However, there are only a few available inorganic layered materials with positive charge, and only two reports of DNA

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Scheme 1. The proposed fabrication and decomposition of a DNA/chitosan/ α -ZrP bioinorganic nanocomposite.

being held by such positively charged layered inorganic materials were found: for the layered double hydroxide (LDH) and aminopropyl-functionalized magnesium phyllosilicate (AMP) [12–15]. On the other hand, the more facile negatively charged layered materials, such as α -ZrP, zirconium phosphonates, montmorillonite, hydroxyapatite and titanate, have been extensively investigated to capture a positively charged protein or enzyme [16–23]. It is understandable that intercalating DNA into these negatively charged layered materials is difficult. The only example of DNA intercalation into a negatively charged support was finished via the heme protein–calf thymus DNA interaction, with the purpose of improving the activity of intercalated hemoglobin [24]. Obviously, intercalating DNA into negatively charged layered materials to form novel bio-nanocomposites is challenging but promising for finding new candidates as DNA vectors.

In this work, herring sperm DNA was intercalated into α -ZrP, a negatively charged layered material in the presence of chitosan at low pH to form a new ternary bioinorganic DNA/chitosan/ α -ZrP nanocomposite. Here, chitosan was adopted as the intermediary to combine the two negatively charged species, since its pK_a of 6.3 makes chitosan positively charged via the amine group protonation at pHs lower than 6.3. Moreover, the excellent biocompatibility and biodegradability of this natural polysaccharide should also be helpful for biological applications [25, 26]. A pH for the medium in the range of 4.5–6.3 should be adopted for this composition, considering the pI (4.0–4.5) of DNA. In addition, the deprotonation of chitosan at high pH values (>6.3) should decrease the positive charge number of chitosan, resulting in the destruction of ternary nanocomposites and DNA release. The proposed reversible assembly of DNA/chitosan/ α -ZrP nanocomposites at different pHs is shown in scheme 1.

2. Experimental details

2.1. Materials

Herring sperm DNA (D3159, approximately 50 bp, referred to as DNA hereafter) was purchased from Sigma Chemical

Co. Deacetylated chitosan was obtained from Nanjing Debao Biochemical Company. Tetrabutylammoniumhydroxide (TBAOH) aqueous solution (10 wt%) was purchased from Shanghai Chemical Reagent Company. All other reagents were of analytical grade. Aqueous solutions were prepared with deionized water (18 M Ω) purified by a MilliQ system.

2.2. Synthesis and delamination of α -ZrP

α -ZrP was prepared following a modified reported procedure [16, 24]. Thus, phosphoric acid (4.79 g) in 75 ml water was added dropwise with stirring to 75 ml of ZrOCl₂ solution containing 2.42 g ZrOCl₂·8H₂O. Then the mixture was heated and kept at 90 °C for 48 h. The white solid obtained via centrifugation was washed with deionized water and acetone, respectively. The resultant α -ZrP solid was then dried overnight at 60 °C.

The delamination of α -ZrP was finished by sonicating 5 ml of aqueous suspension containing α -ZrP (0.1 g) and TBAOH (0.17 g) for 1 h. The final concentration of exfoliated α -ZrP was approximately 20 mg ml⁻¹.

2.3. Preparation of DNA/chitosan/ α -ZrP and chitosan/ α -ZrP nanocomposites

The herring sperm DNA stock solution (60 μ l, 2.5 mg ml⁻¹, 5 mM Tris, 10 mM NaCl, pH 7.2), exfoliated α -ZrP suspension (30 μ l, 20 mg ml⁻¹) and chitosan (150 μ l, 2.0 mg ml⁻¹, 0.1% HAC buffer, pH 5.5) were added in sequence into 2.76 ml of 0.1% HAC buffer solution (pH 5.5) with shaking. After 24 h of equilibration at 25 °C, the sample was separated by centrifugation. The precipitate was washed twice by 0.1% HAC buffer (3 ml \times 2, pH 5.5) to give DNA/chitosan/ α -ZrP composite. Chitosan/ α -ZrP composite was prepared in a similar procedure, with only the herring sperm DNA stock solution replaced by 5 mM Tris-HCl (pH 7.2). The amount of intercalated DNA in the DNA/chitosan/ α -ZrP nanocomposite could be evaluated by determining the retained DNA in the supernatant according to the UV absorbance at 260 nm.

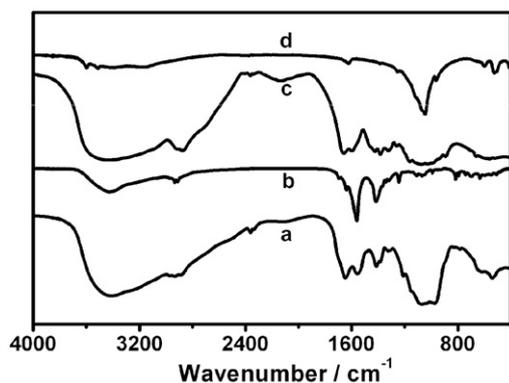


Figure 1. FTIR spectra of DNA/chitosan/ α -ZrP nanocomposite (a), DNA (b), chitosan (c) and α -ZrP (d).

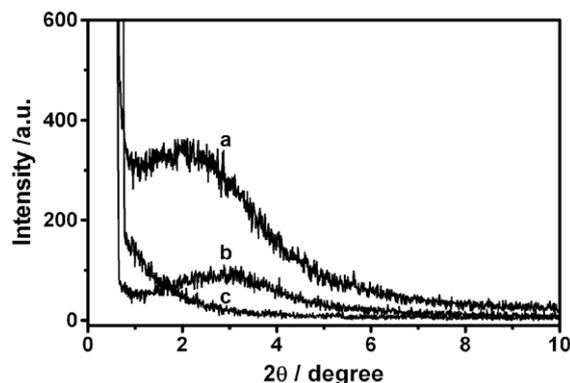


Figure 2. Powder XRD diffraction patterns of DNA/chitosan/ α -ZrP nanocomposite (a), chitosan/ α -ZrP composite (b) and pristine α -ZrP (c).

2.4. DNA release from DNA/chitosan/ α -ZrP nanocomposite

The lyophilized nanocomposite as pellets was transferred carefully into a quartz cuvette, and 3.0 ml buffer solution of different pH was added along the cuvette wall. The release of DNA from the composite (25 °C) was monitored thereafter by recording the UV absorbance of the supernatant at 260 nm. Phosphate buffer solutions (67 mM) with pH 6.5 and 7.0, together with Tris-HCl buffer (50 mM, pH 9.0) were used in this experiment.

2.5. Characterization and measurements

The lyophilized DNA/chitosan/ α -ZrP composite was used for XRD, FTIR and FE-SEM studies. Powder x-ray diffraction patterns were collected on a Philips X'pert x-ray diffractometer. SEM images were obtained with a field-emission scanning electron microscope (FE-SEM; Hitachi S4800). Infrared spectroscopy measurements were recorded on a Bruker Fourier-transform spectrophotometer (Vector 22).

The suspension of lyophilized DNA/chitosan/ α -ZrP composite in 0.1% HAC buffer (pH 5.5) was used for circular dichroism (CD) and UV-vis studies. CD spectra were recorded on a Jasco 810 spectropolarimeter. UV-vis absorption spectra were measured with a Perkin Elmer Lambda 35 spectrophotometer, equipped with a bath/circulation thermostat (Lauda E003).

The zeta-potential was recorded by a Malvern zeta sizer (Nano-Z). All samples were prepared in 6 ml 0.1% HAC buffer (pH 5.5). The concentration of chitosan solution was 0.025 mg ml⁻¹. For the exfoliated α -ZrP, a suspension of 0.05 mg ml⁻¹ was used. The DNA/chitosan sample was prepared by mixing 0.0125 mg ml⁻¹ DNA and 0.025 mg ml⁻¹ chitosan in the buffer, and a chitosan/ α -ZrP sample was obtained similarly by mixing 0.025 mg ml⁻¹ chitosan and 0.05 mg ml⁻¹ α -ZrP in the buffer. The sample of DNA/chitosan/ α -ZrP composite was prepared by mixing 0.0125 mg ml⁻¹ DNA, 0.025 mg ml⁻¹ chitosan and 0.05 mg ml⁻¹ α -ZrP in the buffer.

3. Results and discussion

3.1. Characterization of DNA/chitosan/ α -ZrP nanocomposite

Chitosan with a distinct positive charge and DNA with negative charge are required for the formation of DNA/chitosan/ α -ZrP nanocomposite. According to the pK_a of chitosan and the pI of DNA, a medium with a pH of 5.5 should meet the requirement. The composition of DNA/chitosan/ α -ZrP nanocomposite prepared at pH 5.5 by the procedure described above has been investigated by FTIR measurement. As shown in figure 1, the FTIR absorption spectrum of this solid displays all the characteristic absorbance of DNA, chitosan and α -ZrP. For example, the characteristic bands centered at 1558 and 1411 cm⁻¹ come from the stretching vibrations of pyrimidine in DNA, and the band at 1646 cm⁻¹ can be assigned as the stretching vibration of carbonyl (C=O) in chitosan. The broad band from 3600 to 3000 cm⁻¹ should be attributed to the vibrations of N-H and O-H in chitosan and DNA. The hydrogen bonding and electrostatic interaction between chitosan and DNA may be the origin of the minor deviation from the bands of free DNA and chitosan. Moreover, the intensive and broad band from 1300 to 900 cm⁻¹ can be mainly attributed to the vibration of P-O in α -ZrP. This result reveals that DNA, chitosan and α -ZrP all are involved in the composite and a pH of 5.5 favors the formation of this ternary composite.

XRD patterns of DNA/chitosan/ α -ZrP nanocomposite, chitosan/ α -ZrP composite and pristine α -ZrP are shown in figure 2. The diffraction peak of 2θ for pristine α -ZrP was observed at 11.749°, corresponding to its basal spacing of 0.76 nm (see figure S1 in supporting information, available at stacks.iop.org/Nano/21/105102/mmedia). For the chitosan/ α -ZrP binary composite, the peak of 2θ at 11.749° was no longer observed. In contrast, a low-angle diffraction peak was observed at 2.885°, which corresponds to an interlayer distance of 3.06 nm. Comparison of the interlayer space between α -ZrP and chitosan/ α -ZrP composite reveals a layer distance increment of 2.30 nm. The result implies that more than one layer of chitosan has been intercalated into the interlayer of α -ZrP, since the thickness of one polysaccharide layer is 0.38 nm [27]. DNA/chitosan/ α -ZrP ternary composite,

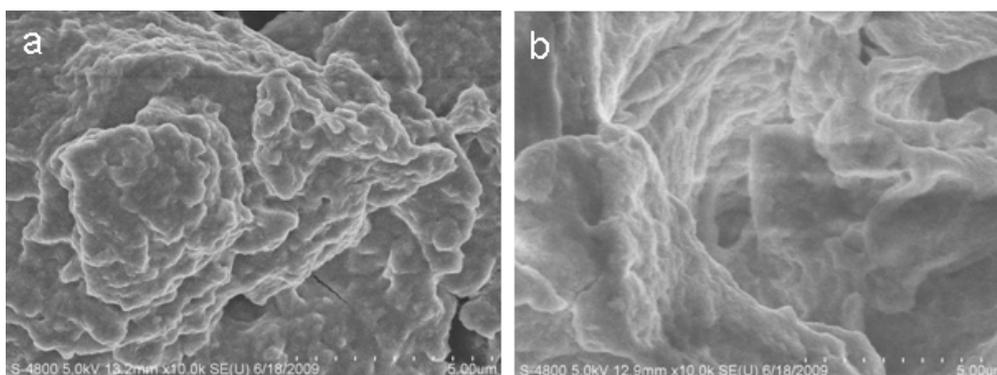


Figure 3. SEM images of pristine α -ZrP (a) and DNA/chitosan/ α -ZrP nanocomposite (b).

however, exhibited a low-angle diffraction peak of even smaller 2θ angle at 2.075° , corresponding to an interlayer distance of 4.25 nm. The interlayer space was increased by 3.49 nm from α -ZrP, which is much larger than the total thickness of one layer of chitosan (0.38 nm) and one layer of DNA (2 nm) [13, 15]. The results imply that the interlayer galleries of α -ZrP may be filled by one layer of DNA and more than one layer of chitosan. The interlayer distance increment from the binary nanocomposite to the ternary nanocomposite is only 1.19 nm, smaller than that from the α -ZrP to the chitosan/ α -ZrP binary composite. Since the thickness of the DNA layer is 2 nm, the current data suggest that chitosan intercalated into the ternary composite might be less than that intercalated into the chitosan/ α -ZrP composite.

The morphologies of pristine α -ZrP and DNA/chitosan/ α -ZrP ternary composite have been further observed by field-emission scanning electron microscopy. SEM images for pristine α -ZrP exhibit the characteristic morphology of a layered structure as expected. Moreover, the layered structure is also prominent in DNA/chitosan/ α -ZrP nanocomposite, as shown in figure 3, even experiencing delamination and guest intercalation.

3.2. Intercalation effect in DNA/chitosan/ α -ZrP composite

To evaluate the effect of intercalation on DNA, the denaturation of intercalated DNA has been investigated by measuring UV absorbance at 260 nm at different temperatures (figures S2–S4, available at stacks.iop.org/Nano/21/105102/mmedia). As shown in figure 4, the absorbance enhancement of free herring sperm DNA occurs initially at 25°C when raising the solution temperature, and the enhancement can still be observed at 85°C . The result suggests that the double-helical structure of this free DNA is unstable and can be partly denatured at low temperature (25°C). For DNA/chitosan, the initial absorbance enhancement was observed when the temperature becomes higher than 30°C , suggesting a slight improvement in DNA stability in the DNA/chitosan complex. However, in the case of DNA/chitosan/ α -ZrP composite, the distinct absorbance enhancement can only be observed when the temperature is raised to 52°C , implying a remarkable improvement of the stability of the double-helical conformation of DNA. The result indicates that the lamellar structure of α -ZrP should be helpful to restrain DNA molecules in the ternary

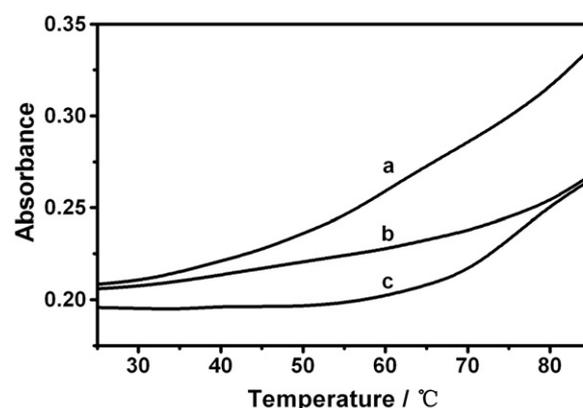


Figure 4. UV absorbance at 260 nm of herring sperm DNA in 0.1% HAC buffer (pH 5.5) at different temperatures: (a) free DNA solution (0.01 mg ml^{-1}), (b) DNA in DNA/chitosan composite (mixture of 0.01 mg ml^{-1} DNA and 0.02 mg ml^{-1} chitosan), (c) DNA intercalated in DNA/chitosan/ α -ZrP composite (suspension of lyophilized solid containing 0.01 mg ml^{-1} DNA).

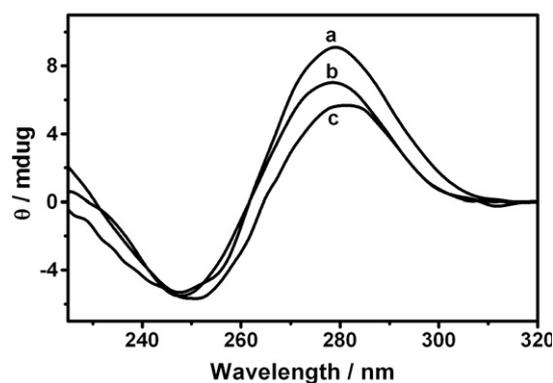


Figure 5. CD spectra of herring sperm DNA in 0.1% HAC buffer (pH 5.5): (a) free herring sperm DNA (0.05 mg ml^{-1}), (b) DNA in DNA/chitosan (mixture of 0.05 mg ml^{-1} DNA and 0.1 mg ml^{-1} chitosan), (c) DNA intercalated in DNA/chitosan/ α -ZrP composite (suspension of lyophilized solid containing 0.05 mg ml^{-1} DNA).

composite, disfavoring adjustment of their conformation at higher temperature.

The conformation of intercalated DNA has been studied by CD spectroscopy. As shown in figure 5, the free herring sperm DNA exhibits a negative band at 247 nm and a positive

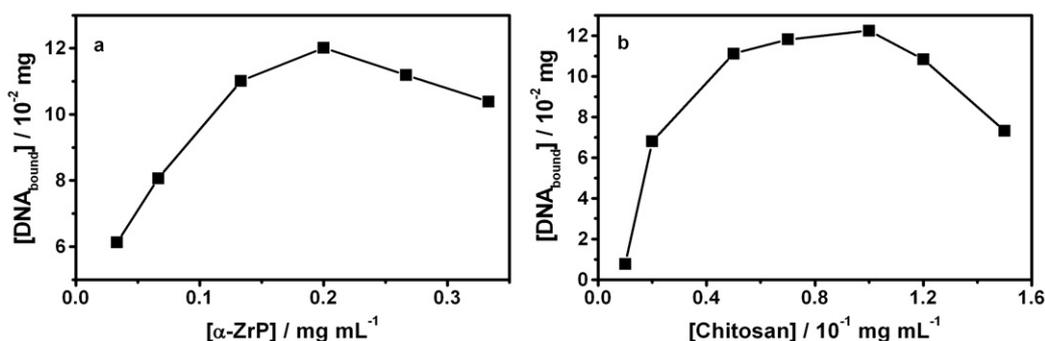


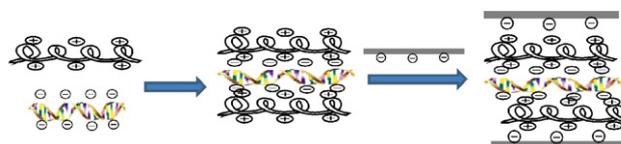
Figure 6. (a) The profile of intercalated DNA when changing the total concentration of α -ZrP at constant $[\text{DNA}]_{\text{total}}$ (0.05 mg ml^{-1}) and $[\text{Chitosan}]_{\text{total}}$ (0.1 mg ml^{-1}). (b) The profile of intercalated DNA when changing the total concentration of chitosan obtained at constant $[\text{DNA}]_{\text{total}}$ (0.05 mg ml^{-1}) and $[\alpha\text{-ZrP}]_{\text{total}}$ (0.2 mg ml^{-1}).

band at 278 nm, confirming the B-type conformation of this free DNA. In addition, DNA in the binary and ternary composites exhibits a very similar negative band to that of free DNA, suggesting that the helical conformation of DNA is not perturbed in the composites [28]. However, the positive band intensity of DNA/chitosan binary composite and DNA/chitosan/ α -ZrP ternary composite is lower than that of free DNA, and DNA in the ternary composite displays the even lower intensity. This phenomenon should be correlated to the perturbation of intercalation with the base stacking in DNA due to the electrostatic interaction involved in composite formation [28]. On the other hand, the weakened hydration of DNA in DNA/chitosan and DNA/chitosan/ α -ZrP composite may also be the reason [29–31].

3.3. DNA binding behavior in composite formation

The DNA binding behavior of chitosan and α -ZrP was investigated by detecting the UV absorbance of the retained DNA in the supernatant separated from the composite formation mixture at 260 nm. At the constant $[\text{Chitosan}]_{\text{total}}$ (0.1 mg ml^{-1}) and $[\text{DNA}]_{\text{total}}$ (0.05 mg ml^{-1}), the intercalated DNA increases quickly with $[\alpha\text{-ZrP}]_{\text{total}}$ when $[\alpha\text{-ZrP}]_{\text{total}}$ is lower than 0.133 mg ml^{-1} . The rate of increase becomes smaller thereafter, and the amount of intercalated DNA attains a maximum when $[\alpha\text{-ZrP}]_{\text{total}}$ being 0.2 mg ml^{-1} . An even higher $[\alpha\text{-ZrP}]_{\text{total}}$ results in a decrease in intercalated DNA (figure 6(a)).

In addition, at constant $[\alpha\text{-ZrP}]_{\text{total}}$ (0.2 mg ml^{-1}) and $[\text{DNA}]_{\text{total}}$ (0.05 mg ml^{-1}), the intercalated DNA increases quickly with $[\text{Chitosan}]_{\text{total}}$ when $[\text{Chitosan}]_{\text{total}}$ is lower than 0.05 mg ml^{-1} . The rate of increase becomes smaller thereafter and maximum intercalation of DNA can be found when $[\text{Chitosan}]_{\text{total}}$ is 0.1 mg ml^{-1} . This means that 82% of the total DNA has been intercalated into DNA/chitosan/ α -ZrP nanocomposite. An even higher $[\text{Chitosan}]_{\text{total}}$ led to a decrease of intercalated DNA (figure 6(b)). The DNA binding behavior was investigated further by zeta-potential measurement. The results show that the zeta-potential values for chitosan, α -ZrP, DNA/chitosan, chitosan/ α -ZrP and DNA/chitosan/ α -ZrP suspensions at pH 5.5 are 28.8, -20.4 , 26.3, 25.1 and -4.62 mV , respectively. These results reveal clearly that



Scheme 2. The proposed self-assembly of DNA/chitosan/ α -ZrP nanocomposite.

chitosan bears net positive charges at pH 5.5, while DNA and α -ZrP bear negative charges. Therefore, both DNA and α -ZrP are able to bind chitosan via electrostatic interaction.

Chitosan is proposed to bind primarily with DNA to form a DNA/chitosan complex, then the positively charged DNA/chitosan complex is intercalated into the interlayer of negatively charged α -ZrP to form the DNA/chitosan/ α -ZrP ternary composite. However, the excessive α -ZrP in the medium will compete with DNA to bind chitosan and decrease the formation of DNA/chitosan composite at higher $[\alpha\text{-ZrP}]_{\text{total}}$. As a result, the intercalated DNA will decrease with $[\alpha\text{-ZrP}]_{\text{total}}$ after the maximum intercalation. The profile of intercalated DNA at different $[\text{Chitosan}]_{\text{total}}$ can also be explained by this mechanism, illustrated as scheme 2, since the excessive chitosan in the medium at higher $[\text{Chitosan}]_{\text{total}}$ will compete with the positively charged DNA/chitosan complex to bind α -ZrP, suppressing the binding of DNA/chitosan complex to form DNA/chitosan/ α -ZrP ternary composite. The positively charged chitosan/ α -ZrP complex might favor the formation of the ternary composite via DNA binding, however, the increase of $[\alpha\text{-ZrP}]_{\text{total}}$ should always disfavor DNA adsorption at constant $[\text{Chitosan}]_{\text{total}}$ and $[\text{DNA}]_{\text{total}}$.

3.4. DNA release from DNA/chitosan/ α -ZrP nanocomposite

For the layer-by-layer assembled bio-nanocomposites, controlled substrate release has been explored by enzymatic degradation [32], hydrolyzation [33] and pH- and salt-induced decomposition [34, 35]. In this work, the DNA release behavior of the DNA/chitosan/ α -ZrP nanocomposite has been investigated via incubation of the lyophilized nanocomposite in buffers of different pH at 25°C , and the release kinetics was

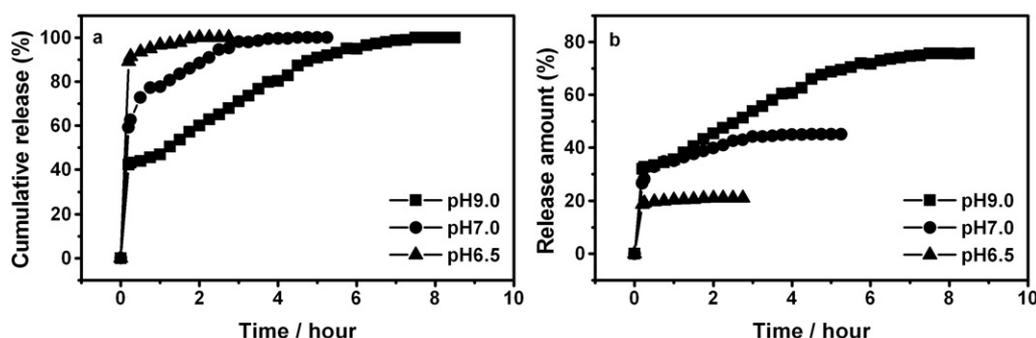


Figure 7. (a) The DNA release kinetics of DNA/chitosan/ α -ZrP nanocomposite at pH 6.5, pH 7.0 and pH 9.0 according to cumulative release. (b) The release percentage of intercalated DNA in DNA/chitosan/ α -ZrP nanocomposite at pH 6.5, pH 7.0 and pH 9.0.

estimated according to the following equation [36]:

$$\text{Cumulative release (\%)} = M_t / M_{\max} \times 100$$

where M_{\max} and M_t is the maximum amount of DNA released and the amount of DNA released at time t , respectively. The released DNA was determined by measuring the UV absorbance of the supernatant separated from the incubation mixture at 260 nm (figures S5 and S6, available at stacks.iop.org/Nano/21/105102/mmedia). As illustrated in figure 7, the release of DNA from DNA/chitosan/ α -ZrP composite is pH-dependent. The maximum DNA release increases with buffer pH and the amount released reaches 21, 45 and 76% of the total intercalated DNA at pH 6.5, 7.0 and 9.0, respectively. The amount of DNA released is almost negligible at pH 6.0. Moreover, there is no detectable release of DNA at pH 5.5, even after 70 h of incubation. The ternary composite exhibits a burst of DNA release in the initial 15 min, and the release rate decreases gradually in the subsequent procedure, with release equilibrium being observed after 2 h at pH 6.5, 4.5 h at pH 7.0 and 7.5 h at pH 9.0, respectively. The effective DNA release at pHs higher than 6.5 suggests that the deprotonation of chitosan amine groups when pH is higher than chitosan pK_a is essential for DNA release. The deprotonation leads to the elimination of the positive chitosan charge, destroying the negative DNA binding to chitosan via electrostatic interaction.

As shown in figure 8, the CD spectrum of the released DNA from the ternary composite is almost identical to that of free herring sperm DNA of same concentration at pH 7.0. A similar case was observed for the DNA released at pH 9.0. All these results suggest that the intercalation and release process do not induce any irreversible change in DNA. This makes the DNA/chitosan/ α -ZrP nanocomposite a suitable candidate DNA vector. Different from the release of DNA from DNA/LDH nanocomposite in intensive acidic media (pH < 2) [13], DNA release from DNA/chitosan/ α -ZrP composite can be realized under neutral conditions. This makes the current system more suitable for application in physiological studies.

4. Conclusion

In this paper, reversible protonation/deprotonation of chitosan amine groups at different pHs was utilized to modulate

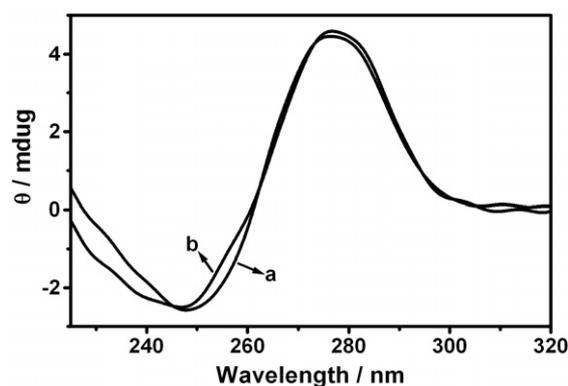


Figure 8. CD spectra of herring sperm DNA (0.02 mg ml^{-1} in 67 mM PBS): (a) DNA released from DNA/chitosan/ α -ZrP nanocomposite at pH 7.0, (b) free herring sperm DNA at pH 7.0.

the interaction between DNA and α -ZrP. At pH 5.5, the protonated chitosan polycations help to mediate the interaction of negatively charged DNA and α -ZrP to form the DNA/chitosan/ α -ZrP bioinorganic nanocomposite without losing the double-helical conformation. Besides the enhanced denaturation temperature of intercalated DNA, the intercalated DNA is able to be effectively released at higher pH. This work provides a simple but effective method to immobilize/release DNA at different pHs. The release of intercalated DNA at physiological pH makes this novel bioinorganic nanocomposite a potential candidate non-viral gene vector. Moreover, this work provides an effective method to intercalate DNA anions into prevalently negatively charged inorganic layered materials and benefits the construction of novel bioinorganic nanocomposites.

Acknowledgments

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References

- [1] Ruiz-Hitzky E, Darder M and Aranda P 2005 *J. Mater. Chem.* **15** 3650
- [2] Choy J-H, Choi S-J, Oh J-M and Park T 2007 *Appl. Clay Sci.* **36** 122
- [3] Kikuchi M, Itoh S, Ichinose S, Shinomiya K and Tanaka J 2001 *Biomaterials* **22** 1705
- [4] Bhatia R B and Brinker C J 2000 *Chem. Mater.* **12** 2434
- [5] Lin F H, Lee Y H, Jian C H, Wong J-M, Shieh M-J and Wang C-Y 2002 *Biomaterials* **23** 1981
- [6] Rosi N L, Giljohann D A, Thaxton C S, Lytton-Jean A K R, Han M S and Mirkin C A 2006 *Science* **312** 1027
- [7] Radu D R, Lai C-Y, Jeftinija K, Rowe E W, Jeftinija S and Lin V S-Y 2004 *J. Am. Chem. Soc.* **126** 13216
- [8] Olton D, Li J, Wilson M E, Rogers T, Close J, Huang L, Kumta N P and Sfeir C 2007 *Biomaterials* **28** 1267
- [9] Liu Z, Winters M, Holodniy M and Dai H 2007 *Angew. Chem. Int. Edn* **46** 2023
- [10] Sokolova V and Epple M 2008 *Angew. Chem. Int. Edn* **47** 1382
- [11] Luo D and Saltzman W M 2000 *Nat. Biotechnol.* **18** 33
- [12] Choy J-H, Oh J-M, Park M, Sohn K-M and Kim J-W 2004 *Adv. Mater.* **16** 1181
- [13] Choy J-H, Kwak S-Y, Jeong Y-J and Park J-S 2000 *Angew. Chem. Int. Edn* **39** 4041
- [14] Desigaux L, Belkacem M B, Richard P, Cellier J, Léone P, Cario L, Leroux F, Taviot-Guého C and Pitard B 2006 *Nano Lett.* **6** 199
- [15] Patil A J, Li M, Dujardin E and Mann S 2007 *Nano Lett.* **7** 2660
- [16] Kumar C V and Chaudhari A 2000 *J. Am. Chem. Soc.* **122** 830
- [17] Bellezza F, Cipiciani A, Costantino U and Nicolis S 2004 *Langmuir* **20** 5019
- [18] Serefoglou E, Litina K, Gournis D, Kalogeris E, Tziaila A A, Pavlidis I V, Stamatis H, Maccallini E, Lubomska M and Rudolf P 2008 *Chem. Mater.* **20** 4106
- [19] Wang L, Nemoto R and Senna M 2004 *J. Eur. Ceram. Soc.* **24** 2707
- [20] Wang Q, Gao Q and Shi J 2004 *J. Am. Chem. Soc.* **126** 14346
- [21] Zhang L, Zhang Q and Li J 2007 *Adv. Funct. Mater.* **17** 1958
- [22] Gao L and Gao Q 2007 *Biosens. Bioelectron.* **22** 1454
- [23] Coradin T, Coupé A and Livage J 2003 *J. Mater. Chem.* **13** 705
- [24] Bhambhani A and Kumar C V 2006 *Adv. Mater.* **18** 939
- [25] Mao H-Q, Roy K, Troung-Le V L, Janes K A, Lin K Y, Wang Y, August J T and Leong K W 2001 *J. Control. Release* **70** 399
- [26] Mansouri S, Lavigne P, Corsi K, Benderdour M, Beaumont E and Fernandes J C 2004 *Eur. J. Pharm. Biopharm.* **57** 1
- [27] Darder M, Colilla M and Ruiz-Hitzky E 2003 *Chem. Mater.* **15** 3774
- [28] Mahadevan S and Palaniandavar M 1998 *Inorg. Chem.* **37** 3927
- [29] Wolf B and Hanlon S 1975 *Biochemistry* **14** 1661
- [30] Hanlon S, Brudno S, Wu T T and Wolf B 1975 *Biochemistry* **14** 1648
- [31] Thyveetil M-A, Coveney P V, Greenwell H C and Suter J L 2008 *J. Am. Chem. Soc.* **130** 4742
- [32] Ren K, Ji J and Shen J 2006 *Biomaterials* **27** 1152
- [33] Wood K C, Boedicker J Q, Lynn D M and Hammond P T 2005 *Langmuir* **21** 1603
- [34] Caruso F, Trau D, Möhwald H and Renneberg R 2000 *Langmuir* **16** 1485
- [35] Schüler C and Caruso F 2001 *Biomacromolecules* **2** 921
- [36] Chen H, He J, Tang H and Yan C 2008 *Chem. Mater.* **20** 5894