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Encapsulation of nucleotides and DNA into Mg–Al layered double hydroxide

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ABSTRACT

The encapsulation of mononucleotides and DNA into Mg–Al layered double hydroxide (LDH, also known as hydrotalcite) by intercalation reaction, and release profile of mononucleotides and DNA was examined. Screening of the intercalation conditions (mononucleotide concentration, reaction temperature, reaction time, and pH) was carried out in order to determine precisely the optimal conditions. Intercalation of all examined mononucleotides and DNA into the chloride form of LDH was found to be possible using the ion-exchange method. The amount of mononucleotide taken up was 0.6–1.5 mmol per 1 g LDH. Intercalation compounds were examined using XRD and solid-state NMR. The interlayer distance of 5′-mononucleotide-intercalated LDH was found to be 14.0–15.3 Å, while that of 3′-mononucleotide-intercalated LDH was 17.4–17.7 Å. Intercalation of double-helix DNA of less than 500 base pairs was verified, with an uptake of 1.8 mmol per 1 g LDH (based on mononucleotide units). The intercalation mechanism and release profile in aqueous K_2CO_3 solution were also investigated. Complete release of the nucleotides was found to take place. The encapsulation makes possible to protect mononucleotides and DNA, and promise the carrier of them to gene.

1. Introduction

Various materials have been examined as carriers for drug delivery systems (DDS), and the idea of a molecular capsule, such as a polymer, liposome, or dendrimer, has attracted much attention (Aulenta et al., 2003). More recently, researchers have examined layered ceramics and other porous compounds as molecular capsules (Nakayama et al., 2003). Hydrotalcite, which has the composition $Mg_6Al_2(OH)_{16}CO_3{\cdot}4H_2O$, is a naturally occurring layered clay (Frondel, 1941; Gastuche et al., 1967). It is a type of double hydroxide, with a structure consisting of a positively charged brucite-like octahedral layer and interlayer anions (Trfiro and Vaccari, 1996). Hydrotalcite-like compounds with a general formula of $M^{2+}{}_{1-x}M^{3+}{}_x(OH)_2(A^{n-})_{x/n}{\cdot}yH_2O$, where M^{2+} and M^{3+} are di- and trivalent metals, respectively, and A^{n-} is the interlayer anion, are generally called layered double hydroxides (LDHs). LDHs have been used in pharmaceutical applications as antacids (Playle et al., 1974) and adsorbents for phosphate (Ookubo et al., 1992, 1994). Recently, the incorporation of organic anions in the interlayer space of LDH – known as intercalation – has been employed in the synthesis of drug–inorganic hybrid materials with the aim of developing molecular capsules (Nakayama et al., 2004).

Mononucleotides are components of DNA. If mononucleotides and DNA can be immobilized in the interlayer space of LDH, this should represent a new type of DDS carrier compound. The pioneering work of Choy et al. on the intercalation of adenosine 5′-phosphate, AMP, and DNA demonstrated the possibility of applying intercalation compounds as gene vectors (Choy et al., 1999, 2000), and further work has been carried out in this regard (Tyner et al., 2004; Xu et al., 2007; Masarudin et al., 2009). In their work, gene's negative charge is compensated with positive charge of LDH layer, and penetration efficiency into the cell is lager than that of other gene vectors. However, detailed examination of the reaction conditions and the physicochemical properties of the intercalation compounds have not yet been carried out. Furthermore, the intercalation of various types of mononucleotide and related compounds has not been examined thus far. In this work, full screening of the intercalation conditions of 5′-GMP, 5′-IMP, 5′-CMP, 5′-UMP and 5′-AMP was carried out, and the physicochemical properties of the intercalation compounds were examined in detail to determine the effect of the base on the intercalation reaction. We also examined the intercalation of 3′-AMP, 3′-GMP, 5′-ADP and 5′-ATP to determine the effect of the phosphorylation position and the mononucleotide charge on intercalation behavior. Intercalation of DNA was examined using the reconstruction method and the ion-exchange method. In order to verify the intercalation compounds as carrier of nucleotides and DNA, the release profiles of mononucleotide and DNA were also examined.

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2. Materials and methods

2.1. Materials

The following layered double hydroxides were purchased from Tomita Chemical Co., Ltd.: $Mg_{0.69}Al_{0.31}(OH)_{2.03}Cl_{0.26}(CO_3)_{0.01}\cdot 0.48H_2O$ (abbreviated as LDH(Cl)), and $Mg_{0.74}Al_{0.26}(OH)_{2.10}(CO_3)_{0.16}\cdot 0.28H_2O$ (abbreviated as LDH(CO_3)). The nucleotides 5′-GMP, 5′-IMP, 5′-CMP, 5′-UMP, 5′-AMP, 5′-ADP, 5′-ATP, 3′-AMP, and 3′-GMP were obtained from Yamasa Co., Ltd. DNA (D-3159) from herring sperm was purchased from Sigma-Aldrich Chemical Co., Ltd. Other chemicals of research grade were purchased from Wako Chemical Co., Ltd. By calcinating 1 g LDH(CO_3) at 500 °C, 0.56 g of calcinated layered double hydroxide (abbreviated as LDH(500)) for the use of reconstruction method was obtained.

2.2. Preparation of mononucleotide- and DNA-intercalated LDH

Intercalation of mononucleotide into LDH was carried out as follows. A powder of the chloride form of layered double hydroxide (LDH(Cl), 1 g), carbonate form of layered double hydroxide (LDH(CO_3), 1 g) or calcinated LDH (LDH(500), 0.56 g) was immersed in distilled water, and the resulting slurry was mixed with 0.100 L of an aqueous solution of mononucleotide (10–200 mmol/L) at several temperatures (0–80 °C) and for various times (1–24 h).

Intercalation of DNA into LDH was carried out using methods based on ion-exchange and reconstruction. LDH(Cl) powder (0.1 g) or LDH(500) powder (0.056 g), was immersed in distilled water. The resulting LDH slurry was mixed with 0.010 L of 0.2 g DNA aqueous solution at several temperatures (0–80 °C) and for various times (1–7 days).

The pH of the reaction solution was adjusted with 6.0 mol/L KOH aqueous solution or HCl aqueous solution. The amount of mononucleotide in the resulting intercalation compounds was determined by elemental analysis using a Sumigraph NC-80 and by spectrophotometric analysis using a Beckman Coulter DU-530 spectrophotometer.

2.3. Deintercalation of mononucleotides

Deintercalation of the mononucleotides was carried out as follows. Powdered mononucleotide-intercalated LDH was immersed in 0.100 L of 5–400 mmol/L K_2CO_3 aqueous solution at 25–60 °C or XIIIth Japanese Pharmacopoeia second fluid (JP XIII 2nd fluid), artificial intestinal juice (pH 6.8). The amount of mononucleotide released was measured by the amount of C in the mononucleotide-intercalated LDH using Sumigraph NC-80 elemental analyzer or by the concentration of mononucleotide in residual solution using Beckman coulter DU-530 spectrophotometer.

2.4. Characterization

Powder X-ray diffraction (XRD) was carried out to monitor the intercalation compounds, using a Rigaku Denki Rint 2000 diffractometer with Ni-filtered Cu$K\alpha$ radiation.

Solid-state ^{31}P and ^{27}Al magic angle spinning (MAS) NMR spectra of the intercalation compounds were obtained using a JEOL GX-270W spectrometer operating at 109.4 MHz for ^{31}P and 70.47 MHz for ^{27}Al. A single pulse sequence with ^{1}H high-power decoupling was used to obtain ^{31}P MAS NMR spectra, which were acquired by accumulating 128 free induction decays, FIDs, with a recycle time of 20 s and a $\pi/2$ pulse of 7.5 μs. A single pulse sequence with a $\pi/2$ pulse of 6.2 μs and ^{1}H high-power decoupling was used for ^{27}Al MAS NMR spectra, which were acquired by accumulating 32 FIDs with a recycle time of 20 s. The MAS rate was 3.0–4.0 kHz. The chemical shift references for ^{31}P and ^{27}Al nuclei were α-$Zr(HPO_4)_2\cdot H_2O$ (−18.7 ppm) and $AlCl_3\cdot 6H_2O$ (0 ppm), respectively.

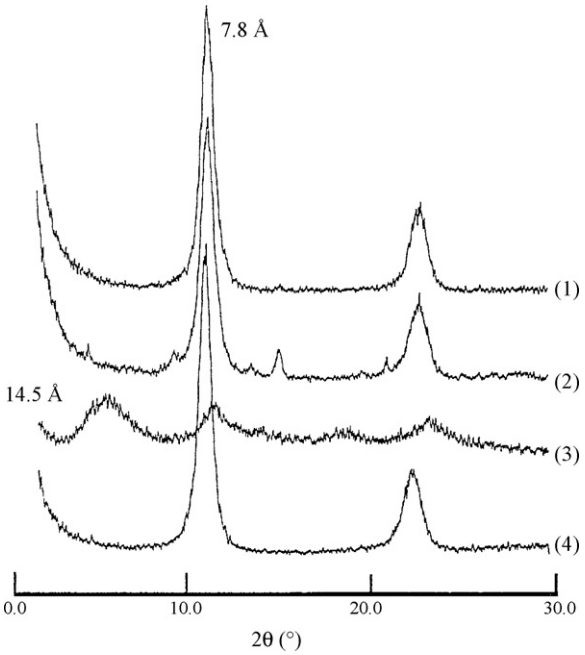

Fig. 1. XRD patterns of (1) 5′-GMP/LDH(500), (2) LDH(CO_3), (3) 5′-GMP/LDH(Cl), and (4) LDH(Cl).

3. Results and discussion

3.1. Intercalation of mononucleotides

The affinity of mononucleotide to various types of LDHs was examined at first using 5′-GMP as reference. Fig. 1 shows the XRD patterns of 5′-GMP-intercalated LDH (abbreviated as 5′-GMP/LDH) synthesized using various types of LDH. A broad peak at 14.5 Å observed for 5′-GMP/LDH(Cl) indicates intercalation of 5′-GMP into LDH(Cl). For the other types of LDH used – LDH(CO_3) and LDH(500) – there was no peak due to intercalation. Therefore, intercalation by the ion-exchange method using LDH(Cl) was concluded to be the optimum method. Thereafter, detail experimental condition was examined only for LDH(Cl).

In order to survey the optimal conditions for intercalation, various reaction conditions, that is, temperature, reaction time, concentration and pH, were examined for the representative mononucleotides 5′-GMP and 5′-IMP. An investigation of temperature dependence showed that the uptake of both mononucleotides did not change between 25 and 60 °C, although a slight decrease in uptake was observed below room temperature. Therefore, 25 °C was concluded to be a suitable temperature for the reaction. Fig. 2 shows the time dependence obtained for uptake of 5′-GMP and 5′-IMP at room temperature. Although the rates of intercalation were different for these two mononucleotides, equilibrium was attained after 5 h for both. The intercalation of 5′-IMP was completed within 1 h. The uptake for both 5′-GMP and 5′-IMP was 1.3 mmol/g. Fig. 3 shows the effect of the initial concentrations of 5′-GMP and 5′-IMP on intercalation. Uptake increased with increasing initial concentration, with saturation at around 50 mmol/L. It was reported previously that saturation occurred at a certain concentration in the case of monolayer exchange of guest anions (Nakayama et al., 2003) – that is, an ion-exchange reaction – whereas Freundlich-type intercalation occurred for neutral guest

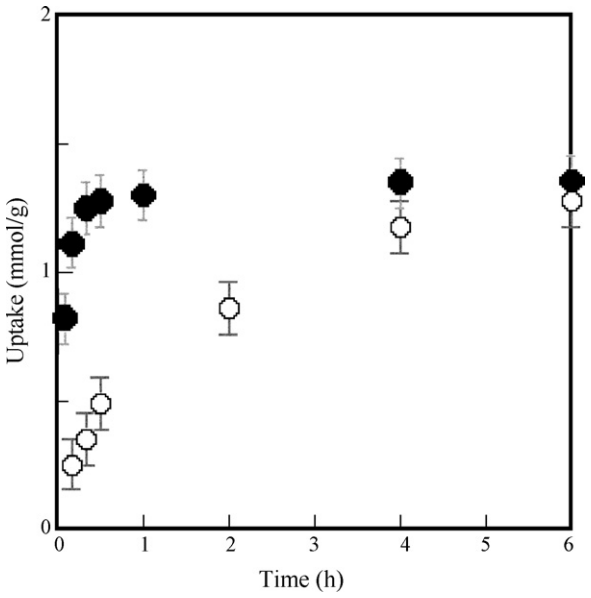

Fig. 2. Time dependence of uptake of 5′-GMP (○) and 5′-IMP (●) into LDH(Cl).

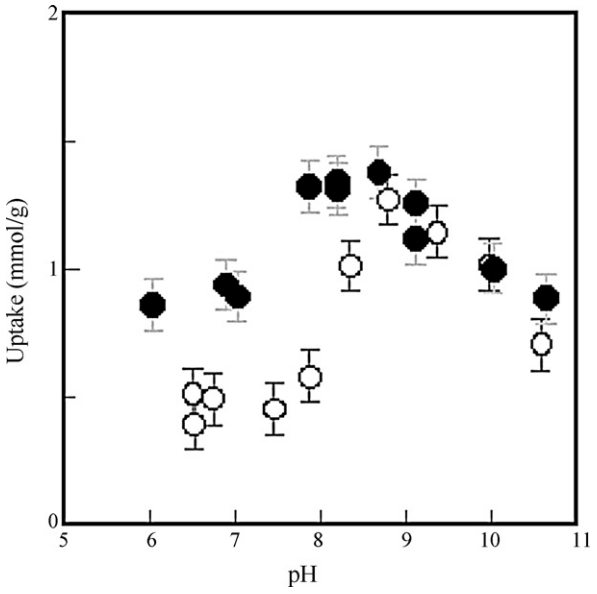

Fig. 4. pH dependence of uptake of 5′-GMP (○) and 5′-IMP (●) into LDH(Cl).

molecules (Nakayama et al., 2004). Therefore, it was concluded that the intercalation of mononucleotides consists of monolayer exchange of mononucleotide anions with chloride anions in the interlayer region. The maximum uptake of 5′-GMP and 5′-IMP was 1.5 and 1.4 mmol/g, respectively, which values are almost half of the ion-exchange capacity, IEC (3.67 mmol/g) of LDH(Cl).

We then examined the effect of pH on the intercalation reaction. Because mononucleotides contain phosphate groups, several different anionic species may exist depending on the pH of the aqueous mononucleotide solution (Ookubo et al., 1994). Fig. 4 shows the pH dependence of the uptake of 5′-GMP and 5′-IMP. Because dissolution of LDH occurs below pH 2, the experiment was performed at pH 6–11. Although the pH dependence was different for the two mononucleotides, the maximum uptake was observed to be 1.4 mmol/g around pH 8–9. It has been reported that the maximum uptake of acidic molecules takes place at pH 6–8, and at higher pH affinity decreases drastically (Nakayama et al., 2003). Although 5′-GMP exists mainly as monovalent anions at pH 6, the uptake was less than 0.5 mmol/g at this level, probably due to low affinity, as mentioned above. The situation is the same for other mononucleotides. Based on these experiments, it was concluded that the optimal intercalation conditions of 5′-GMP are as follows: a starting concentration of 50 mmol/L, room temperature, pH 8, and 5 h.

The mononucleotides, which have monophosphate, diphosphate and triphosphate, are expected to show different intercalation behavior, because anionic charges of mononucleotides are different. Fig. 5 shows the uptake of 5′-AMP, 5′-ADP, and 5′-ATP into LDH over various reaction times. The equilibrium times for intercalation were 30 min, 1 h and 10 min, respectively; it may be noted that the intercalation reaction was particularly fast for 5′-ATP (10 min). It has been reported that affinity with LDH is higher for anions with a greater charge (Miyata, 1983), and this is thought to be reason for the fast intercalation of 5′-ATP. The

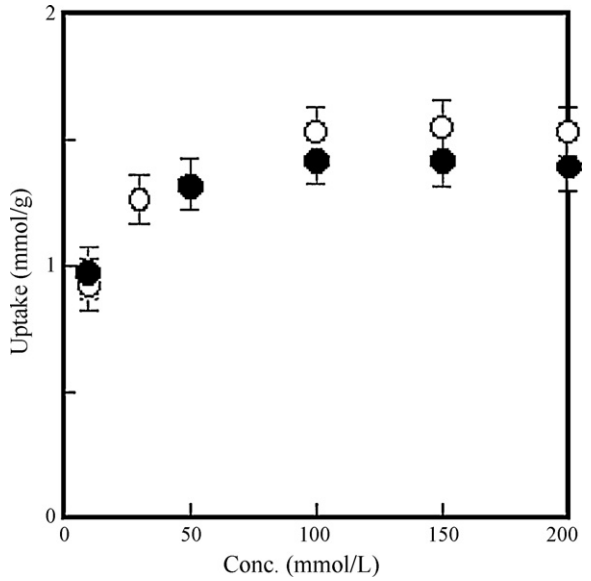

Fig. 3. Concentration dependence of uptake of 5′-GMP (○) and 5′-IMP (●) into LDH(Cl).

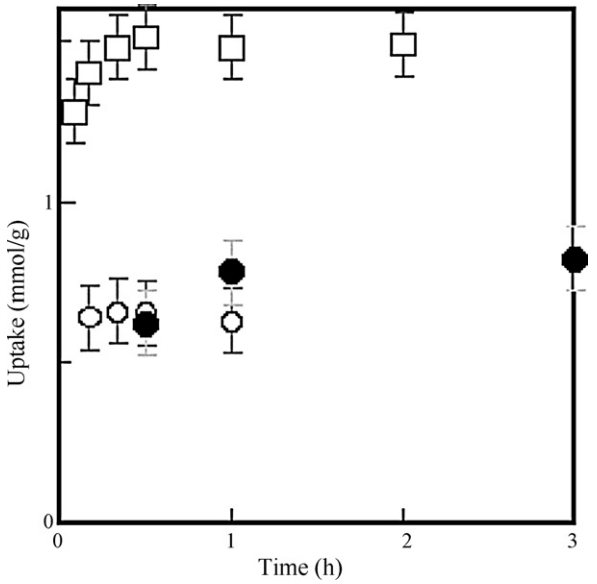

Fig. 5. Time dependence of uptake of 5′-AMP (□), 5′-ADP (●) and 5′-ATP (○) into LDH(Cl).

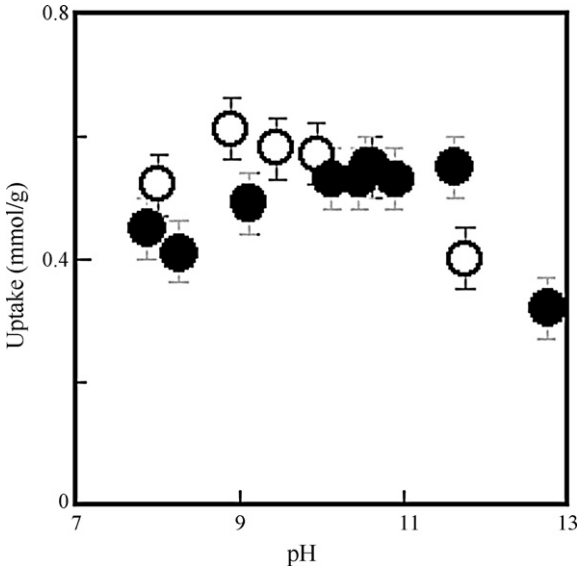

Fig. 6. pH dependence of uptake of 5′-ADP (●) and 5′-ATP (○) into LDH(Cl).

uptake for 5′-ADP and 5′-ATP was 0.7 and 0.6 mmol/g, respectively, due to the larger anionic charge of these anions. Fig. 6 shows the pH dependence of 5′-ADP and 5′-ATP uptake; the maximum uptake was observed at pH 9–10 for 5′-ADP and at pH 10–11 for 5′-ATP.

The intercalation conditions for the other mononucleotides were also examined. The mononucleotide/LDH interlayer distance and the uptake at the optimal reaction conditions are summarized in Table 1. The ion-exchange reaction was completed within 5 h for all nucleotides; it was concluded that the intercalation of nucleotides is particularly fast. For all mononucleotides except 5′-ADP and 5′-ATP, the optimal pH was found to be 8. Because the pK_1, pK_2 and pK_3 of 5′-GMP are 0.7, 2.4 and 6.7, respectively (Stenesh, 1998), more than 90% of 5′-GMP molecules exist as divalent anions at pH 8. The theoretical uptake of 5′-GMP as divalent anions is 1.83 mmol/g, which is close to the uptake observed at pH 8. Therefore, it was concluded that 5′-GMP is intercalated as divalent anions at pH 8. Based on the same reason, it was concluded that the other mononucleotides – except for 5′-ADP and 5′-ATP – were also intercalated as divalent anions, although the measured exchange ratio was low for 3′-mononucleotides. Based on the pK_a values of 5′-ADP and 5′-ATP, they are thought to exist as tri- and tetravalent anions, respectively, at the optimal reaction conditions. The theoretical uptake of 5′-ADP and 5′-ATP as tri- and tetravalent anions is 1.2 and 0.9 mmol/g, respectively, which is close to the observed uptake of both anions, as shown in Table 1. Therefore, it was concluded that 5′-ADP and 5′-ATP were intercalated as trivalent and tetravalent anions, respectively. The exchange ratios for both nucleotides were 70%. The low exchange ratios compared with that of 5′-AMP are thought to be due to their size and arrangement in the interlayer space. We will return to this point later.

Table 1
Data of mononucleotide/LDHs.

Nucleotide	Conditions		Uptake	d
	Time (h)	pH	(mmol/g)	(Å)
5′-GMP	5	8	1.4 ± 0.1	14.5
5′-IMP	1	8	1.4 ± 0.1	14.0
5′-CMP	5	8	1.1 ± 0.1	14.0
5′-UMP	1	8	1.2 ± 0.1	14.0
5′-AMP	1	8	1.5 ± 0.1	15.3
5′-ADP	1	9–10	0.8 ± 0.05	15.3
5′-ATP	1	10–11	0.6 ± 0.05	15.3
3′-AMP	5	8	1.1 ± 0.1	17.7
3′-GMP	5	8	1.1 ± 0.1	17.4

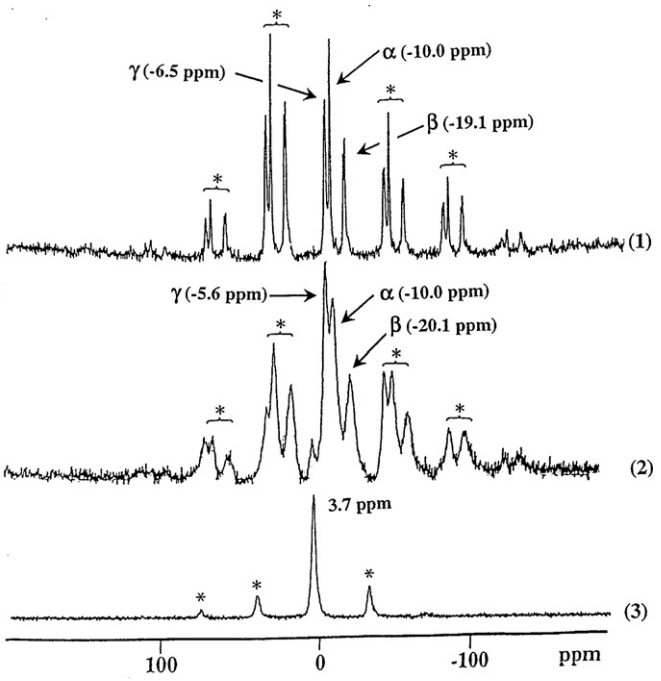

Fig. 7. ^{31}P MAS NMR spectra of (1) 5′-ATP, (2) 5′-ATP/LDH, and (3) 5′-AMP/LDH. *Denotes spinning side bands.

3.2. Characterization of intercalation compounds

In the case of 5′-ADP and 5′-ATP, it was thought that hydrolysis of the di- and triphosphate might occur during the intercalation reaction. In order to exclude this possibility, the ^{31}P NMR spectrum of 5′-ATP/LDH was obtained, as shown in Fig. 7. 5′-ATP itself show three ^{31}P NMR signals of triphosphate group at −6.5, −10.0 and −19.1 ppm. In 5′-ATP/LDH three peaks at −5.6, −10.0 and −20.1 ppm were observed almost similar chemical shift values to 5′-ATP itself. Therefore, it suggests that almost no hydrolysis of triphosphate had taken place. The same result was obtained for 5′-ADP/LDH. Therefore, it was concluded that 5′-ADP and 5′-ATP were intercalated into LDH without decomposition.

As shown in Table 1 the interlayer distances of the 5′-mononucleotides – except for 5′-AMP, 5′-ADP and 5′-ATP – were 14.0–14.5 Å, although the base sizes are different for 4 mononucleotides, that is, 5′-GMP, 5′-IMP, 5′-CMP and 5′-UMP. The ^{31}P MAS NMR spectra of 5′-mononucleotide/LDH showed a single peak at −4.2 ppm, which is almost similar chemical shift values of 5′-mononucleotides itself, suggesting that no decomposition of the 5′-mononucleotides had taken place during the intercalation reaction. The ^{27}Al MAS NMR spectra of 5′-mononucleotide/LDH showed a single peak at −9.2 ppm due to the LDH framework, which is close to the spectrum of LDH(Cl); this suggests that there was no deformation of the LDH framework. Based on these results, it is suggested that the intercalation of 5′-mononucleotides proceeds without decomposition. Considering the molecular sizes and shapes of the 5′-mononucleotides, it is expected that 5′-mononucleotides arrange themselves as shown in Fig. 8.

As shown in Table 1 the interlayer distances for 5′-AMP, 5′-ADP and 5′-ATP/LDH were all 15.3 Å. This fact suggests that the long axis of di- or triphosphate is parallel to the LDH layer. The attractive force between the positive charge of the LDH layer and negative charge of the guest species is strongest in this parallel arrangement.

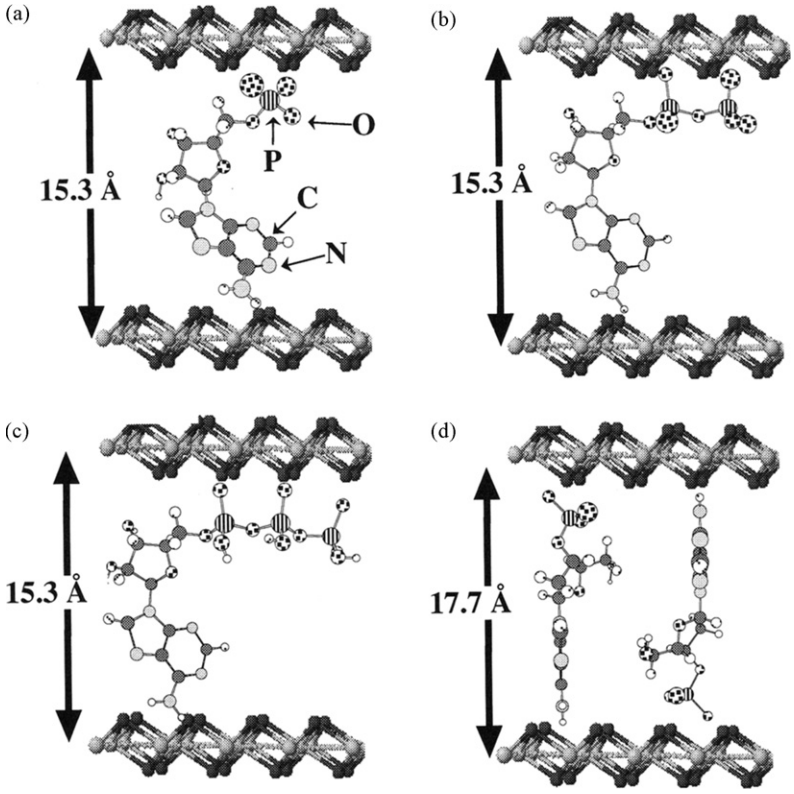

Fig. 8. Schematic structural models of (a) 5′-AMP/LDH, (b) 5′-ADP/LDH, (c) 5′-ATP/LDH and (d) 3′-AMP/LDH.

The low exchange ratio for 5′-ADP and 5′-ATP are thought to be due to low packing of these nucleotides in the interlayer space, as shown in Fig. 8.

The interlayer distances for 3′-mononucleotides were 17–18 Å and their uptake was 1.1 mmol/g (Table 1). In comparison with the 5′-mononucleotides, an expansion of the interlayer distance and a decrease in uptake were observed. In the normal intercalation reaction, expansion of the interlayer distance is accompanied by an increase in uptake. However, this is not the case for 3′-mononucleotides, which is probably due to their molecular shape.

3.3. Intercalation of DNA

After our observation that various mononucleotides were easily intercalated into LDH(Cl) by the ion-exchange method, we examined the intercalation of DNA under various conditions. DNA from herring sperm (Sigma-Aldrich) was used for the experiment. The absorbance ratio A_{260}/A_{280} at pH 8.5 was 2.00, which suggested that the DNA was highly pure. Therefore, we used this crude DNA without removal of protein. In order to determine the size effect of DNA for the intercalation reaction, DNA cutting was performed using a syringe, and the size was analyzed by agarose gel electrophoresis. Fig. 9 shows the electrophoresis diagram of DNA after various cutting processes as listed in Table 2. The samples showed bands of 100–500 bp. The various cutting processes resulted in no significant change in DNA size.

The intercalation of DNA processed using various cutting procedures into LDH(Cl) was carried out at 80 °C for 4 days. Expansion of the interlayer distance was observed at any DNA with various cutting process, as summarized in Table 3. For the reaction carried with no pH adjustment, the XRD pattern showed a sharp peak at 23.0 Å and the uptake was 1.7 mmol/g, whereas for that at pH 9.0 the XRD pattern showed a broad peak at 16 Å and the uptake was 1.4 mmol/g. Therefore, intercalation of DNA was found to depend

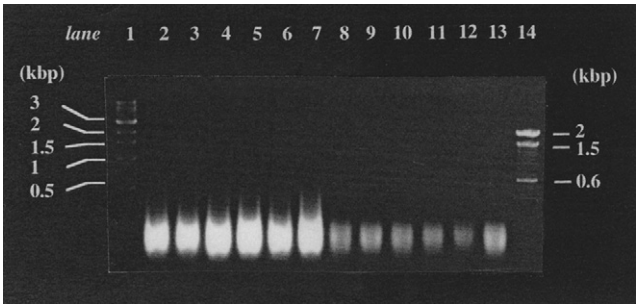

Fig. 9. Analysis of DNA size by agarose gel electrophoresis for lanes 1–14.

Table 2
Shering condition of DNA molecules.

Lane	pH	G[a]	Times[b]
1	1 kbp DNA marker		
2	9.0	No shering	
3		22	5
4			15
5			30
6		18	15
7		22	15
8	7.3	No shering	
9		22	5
10			15
11			30
12		18	15
13		22	15
14	100 bp DNA marker		

[a] Gauge of hypodermic needle.
[b] Time of passing rapidly through hypodermic needle.

Table 3
Uptake of DNA and interlayer distance of DNA/LDH.

G	Times	pH	Uptake (mmol/g)	*d* (Å)
22	5	7.3	1.7 ± 0.1	23.0
	15		1.6 ± 0.1	
	30		1.7 ± 0.1	
18	15		1.8 ± 0.1	
22	5	9.0	1.3 ± 0.1	16.0
	15		1.3 ± 0.1	
	30		1.4 ± 0.1	
18	15		1.4 ± 0.1	

largely on the pH of reaction solution, and the reaction with no pH adjustment gave better results. Thereafter, uncut DNA was used in the experiments, without pH adjustment.

Fig. 10 shows the uptake of DNA at various reaction temperatures. Higher reaction temperatures resulted in increased uptake, with the maximum uptake observed at 80 °C. Compared with the mononucleotides, a higher temperature was essential for the intercalation of DNA. Although DNA has a considerable negative charge at pH 7.3, the larger size of DNA prevents smooth intercalation at room temperature. Actually the intercalation proceeds by ion-exchange mechanism, and the charge density of mononucleotide and DNA is comparable. However, in the exchange process, diffusion of long flexible chain of DNA within the limited interlayer space will be rate-determining step of the intercalation. So higher temperature is essential for the intercalation of DNA. The ^{31}P MAS NMR spectrum of DNA/LDH showed a single peak at −0.3 ppm, which is almost the same as the signal of DNA itself, suggesting that no decomposition of DNA had taken place during the intercalation reaction. The ^{27}Al MAS NMR spectrum of DNA/LDH showed a single peak at −9.0 ppm due to the LDH framework. This value is close to that of LDH(Cl), which implies that no deformation of the LDH framework takes place on intercalation of DNA. Based on the size of double-helix DNA, it is suggested that intercalation takes place as shown in Fig. 11. The XRD pattern of DNA/LDH(500) synthesized by reconstruction method showed a broad peak at 18 Å, with an uptake of 0.4 mmol/g. Therefore, intercalation of DNA by the reconstruction method was possible, although uptake of DNA was low compared with ion-exchange method. Therefore, the ion-exchange method is useful for DNA intercalation. The uptake of DNA was

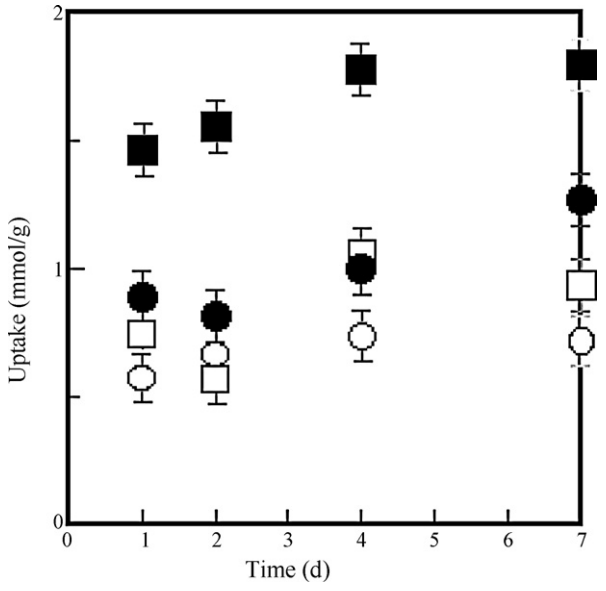

Fig. 10. Time dependence of uptake of DNA into LDH(Cl) at 25 °C (□), 40 °C (○), 60 °C (●), and 80 °C (■).

Table 4
Release of mononucleotides from mononucleotide/LDH.

Nucleotide	Amount of guest (mmol/g mononucleotide/LDH)	Release (%)
5′-GMP	1.4 ± 0.1	91
5′-IMP	1.4 ± 0.1	89
5′-CMP	1.1 ± 0.1	90
5′-UMP	1.2 ± 0.1	76
5′-AMP	1.5 ± 0.1	81
5′-ADP	0.8 ± 0.05	65
5′-ATP	0.6 ± 0.05	65
3′-AMP	1.1 ± 0.1	85
3′-GMP	1.1 ± 0.1	85

1.8 mmol/g based on mononucleotide units, which is half of the ion-exchange capacity. Therefore, the phosphate charge of DNA per mononucleotide unit should be −2, which is similar to mononucleotide intercalation. Almost complete intercalation of DNA was achieved.

3.4. Deintercalation of mononucleotides and DNA

In order to investigate the possibility of using intercalated LDH as a drug delivery system (DDS) host, that is, release of mononucleotides and DNA, deintercalation of mononucleotide/LDH and DNA/LDH was examined. A time dependence experiment for 5′-GMP/LDH showed that the deintercalation reaction was completed within 3 h. There was no temperature effect between 25 and 80 °C. Fig. 12 shows the release profile of 5′-GMP in K_2CO_3 aqueous solution. Saturation was observed at 200 mmol/L aqueous K_2CO_3, and the release ratio was 91%. Deintercalation reactions for the other mononucleotides were performed in a similar fashion, and the results are summarized in Table 4. For 5′-GMP, 5′-CMP, 5′-UMP, 5′-AMP, 3′-AMP, 3′-GMP, the release ratios were 80–90%, while the release ratios for 5′-ADP and 5′-ATP were less than 70%. The large anionic charges of 5′-ADP and 5′-ATP are thought to be responsible for the low release ratios. After deintercalation reaction the ^{31}P MAS NMR signal of 5′-GMP at −4.2 ppm was reduced considerably, suggesting almost complete release of 5′-GMP from 5′-GMP/LDH, and a sharp ^{27}Al NMR signal at 9 ppm (LDH) remained after the deintercalation reaction, suggesting no decomposition of LDH host layer. Furthermore, XRD pattern after deintercalation reaction is exactly the same as that of LDH(CO_3). Therefore, it was concluded that 5′-GMP was exchanged with CO_3^{2-} anions in the deintercalation reaction. This result shows that the intercalation and release of mononucleotides are reversible.

The deintercalation of DNA from DNA/LDH was also examined. A time dependence experiment for DNA/LDH showed that the deintercalation reaction was completed within 2 h at 25 °C, and in less than 30 min at 60 °C. The slow deintercalation reaction compared with that of mononucleotide/LDH is thought to be due to the large charge of DNA. A concentration experiment showed saturation at 90% in the deintercalation reaction at 60 °C (1 h). Experiments similar to those carried out for 5′-GMP (XRD and ^{27}Al NMR) showed that DNA was exchanged with CO_3^{2-} anions in the deintercalation reaction. Fig. 13 shows the release profile of DNA in XIIIth Japanese Pharmacopoeia second fluid (JP XIII 2nd fluid), artificial intestinal juice (pH 6.8). The reaction to release DNA was completed within 24 h, and release ratios were 30, 60, and 70% at 20, 37, and 60 °C, respectively. The low release ratio is thought to be due to the low affinity of phosphate with JP XIII 2nd fluid compared with CO_3^{2-}.

This result shows that reversible intercalation and release of DNA are possible using K_2CO_3 aqueous solution, and that release in JP XIII fluid is also possible.

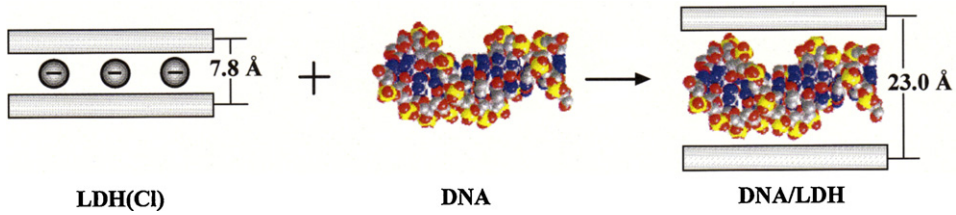

Fig. 11. Schematic illustration of DNA intercalated into LDH(Cl).

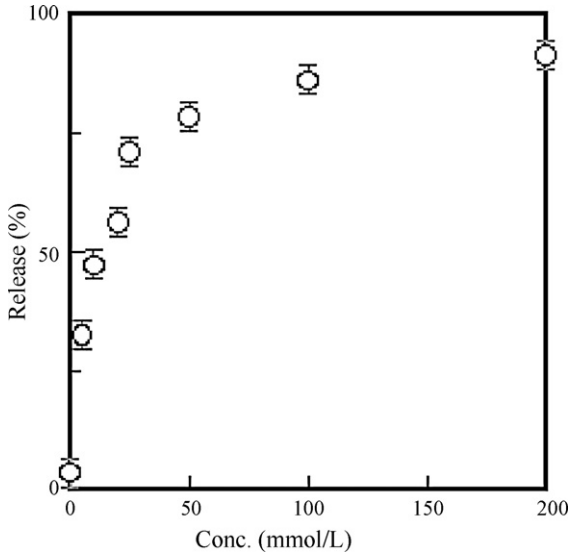

Fig. 12. Release profile of 5′-GMP from 5′-GMP/LDH in K_2CO_3 aqueous solution at various concentrations.

3.5. General discussion

As mentioned in Section 1, Choy et al. showed that the intercalation compound of adenosine 5′-phosphate, AMP, and DNA with LDH can be used as gene vectors (Choy et al., 1999, 2000). In this work we could successfully show that various kind of mononucleotides could also intercalated into LDH, and also show the complete reaction conditions for these intercalation compounds for the first time. Especially, pH was shown to be critical for the intercalation reaction. Combined with Choy work, all the mononucleotides and related compounds also can be transferred in gene in the similar way.

Detail characterizations were performed for these intercalation compounds of mononucleotides by uptake, XRD, and Solid-state NMR. And we also can show the different intercalation behaviors of 5′-monophosphate, 3′-monophosphate, diphosphate and triphosphate for the first time.

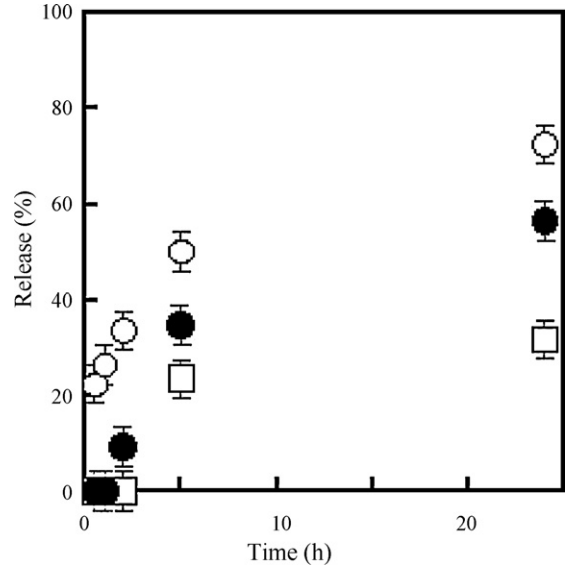

Fig. 13. Release profile of DNA from DNA/LDH in JP XIII 2nd fluid at 20 °C (○), 40 °C (●), and 60 °C (□).

4. Conclusion

Nucleotides and DNA were intercalated into LDH by an ion-exchange method using LDH(Cl). The amount of nucleotide in the intercalation compounds was around 1.5 mmol per 1 g of LDH(Cl). All of the mononucleotides examined, except for 5′-ADP and 5′-ATP, were found to exist as divalent anions in the interlayer region of LDH. Therefore, almost all the nucleotides and DNA examined in this work were verified to be immobilized in the LDH layer, and these intercalation compounds would be attractive candidates of nucleotides and DNA carrier.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2010.04.013.

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