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Intercalation of amino acids and peptides into Mg–Al layered double hydroxide by reconstruction method

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Abstract

The intercalation of amino acids and some peptides into Mg–Al layered double hydroxide known as hydrotalcite was examined. Although the intercalation by ion-exchange method was unsuccessful, all the amino acids except for Lys and Arg, and peptides examined could be intercalated into the layered double hydroxide by reconstruction method using Mg–Al oxide precursor. The uptake amounts of amino acids and peptides were 0.9–2.7 mmol per 1 g of LDH. Intercalation compounds were examined by using XRD and solid-state NMR. For Gly, Ala, Ser, Thr, Pro, Asn, Gln, Asp, Glu, and aspartame the intercalation accompanied the expansion of interlayer distance of the solid products, whereas the other amino acids and oligoglycine showed no expansion. The intercalation mechanism and release profile in K_2CO_3 aqueous solution were also investigated. And the cointercalation of amino acids and peptides into Mg–Al LDH and easy release of amino acids from the LDH layer were found.

Keywords: Intercalation; Amino acid; Layered double hydroxide; Hydrotalcite; Solid-state NMR

1. Introduction

Hydrotalcite is naturally occurring layered clay with the composition of $Mg_6Al_2(OH)_{16}CO_3{\cdot}4H_2O$ (Frondel, 1941; Gastuche et al., 1967). Hydrotalcite is a sort of double hydroxides, and its structure consists of positively charged brucite-like octahedral layer and negatively charged anions in the interlayer space (Trfiro and Vaccari, 1996). Hydrotalcite-like compounds with a general formula of $M_{1-x}{}^{2+}M_x{}^{3+}(OH)_2(A^{n-})_{x/n}{\cdot}yH_2O$, where M^{2+} and M^{3+} are di- and tri-valent metals, respectively, and A^{n-} is interlayer anion, are generally called layered double hydroxide (LDH), which is easily produced by the co-precipitation of two metal salts. LDH has been used as inorganic anion-exchanger and base-catalyst in the chemical industry (Cavani et al., 1991), and as an antacid (Playle et al., 1974) and an adsorbent of phosphate (Ookubo et al., 1994; Ookubo et al., 1992) in pharmaceutical application. Recently, organic and inorganic hybrid materials based on layered compounds have also been focused the spotlight of attention as materials with new functions (Newman and Jones, 1998; Dutta and Robins, 1994). Incorporation of organic anions in the interlayer space by ion-exchange mechanism, so called, intercalation phenomena, was used for the synthesis of organic–inorganic hybrid materials. LDH has been expected as carrier of drug delivery system (DDS), and DNA-intercalated LDH was reported as gene carrier into cell (Choy et al.,

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1999; Choy et al., 2000). This is because the guest DNA molecule can be stabilized in the interlayer space and recovered by de-intercalation reaction without change of the host compound.

Amino acids are components of enzymes and natural proteins. If enzymes and proteins can be immobilized in the interlayer space of LDH, new type of catalytic active compound with selective reactivity and DDS carrier compound can be expected. In principle, intercalation into LDH occurs by ion-exchange mechanism. DNA is anionic macromolecule and is expected to be intercalated by ion-exchange method (Choy et al., 1999; Choy et al., 2000). On the other hand, amino acids exist as zwitterions and are neutral electrically at around pH 7. Therefore, the intercalation of amino acids and protein are expected to be difficult. By using the coprecipitation method and reconstruction method, it is sometimes possible to intercalate neutral molecules, which could not be intercalated by ion-exchange method (Narita, 2001). Recently, Aizawa et al. reported the intercalation of some amino acids into Zn–Al and Mg–Al LDHs by the coprecipitation method (Aisawa et al., 2001). Although Mg–Al LDH is biocompatible and is used as antacid commercially, the application of Mg–Al LDH as the matrix for controlled release using intercalation is few (Ambrogi et al., 2001). In the present work, in order to consider the possibility of immobilization of protein and enzyme, the intercalation reaction of amino acids and some peptide into Mg–Al LDH by the reconstruction method was examined. Furthermore, the release profile of amino acids and some peptides were examined as well.

2. Materials and methods

2.1. Materials

Layered double hydroxides, $Mg_{0.69}Al_{0.31}(OH)_{2.03}Cl_{0.26}(CO_3)_{0.01}\cdot 0.48H_2O$ (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)) were purchased from Tomita Chemical Co., Ltd. Amino acids and glycylglycine were obtained from Wako Chemical Co., Ltd. Glycylglycylglycine and glycylglycylglycylglycine were purchased from Tokyo Kasei Co., Ltd and Sigma–Aldrich Chemical Co., Ltd, respectively. Ninhydrin reagent was obtained from Beckman Coulter Co., Ltd. The other chemicals of research grade were purchased from Wako Chemical Co., Ltd.

2.2. Preparation of amino acid and peptide-intercalated LDH

Intercalation of amino acid into LDH was carried out as follows. The powder of 1 g of layered double hydroxide (chloride form LDH(Cl) or carbonate form LDH(CO_3)) or 0.56 g calcinated LDH was immersed in the distilled water. The calcination of 1 g LDH(CO_3) was performed at 500 °C for 5 h to form 0.56 g calcinated LDH. Then 100 ml of 1–160 mmol l^{-1} amino acid aqueous solution and the above LDH slurry were mixed at several temperatures and times. The pH of the reaction solution was adjusted by 6.0 mol l^{-1} KOH aqueous solution or HCl aqueous solution. The amounts of amino acid and peptide in the intercalation compounds obtained were determined by the elemental analysis using a Sumigraph NC-80 and by ninhydrin colorimetric method using Beckman coulter DU-530 spectrophotometer (Moore and Stein, 1948).

2.3. Deintercalation of amino acid and oligopeptide

Deintercalation of amino acid from amino acid-intercalated LDH was carried out as follows. The powder of 1 g amino acid-intercalated LDH was immersed in 100 ml of 25 mmol l^{-1} K_2CO_3 aqueous solution at room temperature. The release amount of amino acid was measured by the elemental analysis or spectrophotometry. The deintercalation of peptide was performed by the simlar way.

2.4. Characterization

Powder X-ray diffraction (XRD) pattern was measured to monitor the intercalation compound with a Rigaku Denki Rint 2000 diffractometer using Ni-filtered Cu Kα radiation.

The solid-state ^{13}C cross-polarization and magic angle spinning (CP/MAS) and ^{27}Al magic angle spinning (MAS) NMR spectra of the intercalation compounds were obtained using a JEOL GX-270W spectrometer operating at 67.9 MHz for ^{13}C nucleus and 70.4 MHz for ^{27}Al nucleus, respectively. Cross-polarization (CP) pulse sequence with ^{1}H high-power decoupling was

used to obtain ^{13}C CP/MAS NMR spectra, which were acquired by accumulating 600–1200 FID's with a recycle time of 4 s and a contact time of 1 ms. Single pulse sequence with a $\pi/2$ pulse of 6.2 μs with ^{1}H high-power decoupling was used for ^{27}Al MAS NMR spectra, which were acquired by accumulating 256 FID's with a recycle time of 20 s. The MAS rate was 4.0–4.5 kHz. Chemical shift references for ^{13}C and ^{27}Al nuclei are TMS and $AlCl_3{\cdot}6H_2O$, respectively.

3. Results and discussion

3.1. Intercalation of amino acids

In order to survey the optimal intercalation condition of amino acids into LDH (abbreviated as LDH/amino acid), various reaction conditions were examined for leucine (Leu) and phenylalanine (Phe) as example. It was impossible to intercalate these amino acids by the ion-exchange method using LDH(Cl). It is well-known that neutral molecules, which could not be intercalated by ion-exchange method, could sometimes be intercalated by using the coprecipitation method and the reconstruction method (Trfiro and Vaccari, 1996). Fig. 1 shows XRD patterns of Leu-intercalated LDH synthesized using calcinated LDH(CO_3) at different temperature (LDH(*T*)/Leu where *T* shows the calcination temperature (°C)). Due to the ineffective calcination, the intercalation of Leu did not occur below 300 °C at all. A sharp peak (◆) at 14.6 Å observed for LDH(500)/Leu and LDH(600)/Leu indicates the intercalation of Leu into LDH. It is well known that the calcination at higher temperature of over 700 °C induces phase separation and the original layered structure does not reconstruct any more (Ulibarri et al., 1994). Therefore, the intercalation using LDH(CO_3) calcinated above 700 °C was not effective as shown in Fig. 1. Because the amount of Leu in LDH(500)/Leu and LDH(600)/Leu are almost the same, we used 500 °C as calcination temperature.

The intercalation reaction has been normally performed under N_2 atmosphere to avoid the contamination of CO_2 in atmosphere (Trfiro and Vaccari, 1996). However, for the practical use it is profitable if this operation is omitted. Fig. 2 shows the uptakes of Phe into LDH(500) under air and N_2 atmosphere, respectively. Although the rate of uptake was slow under air,

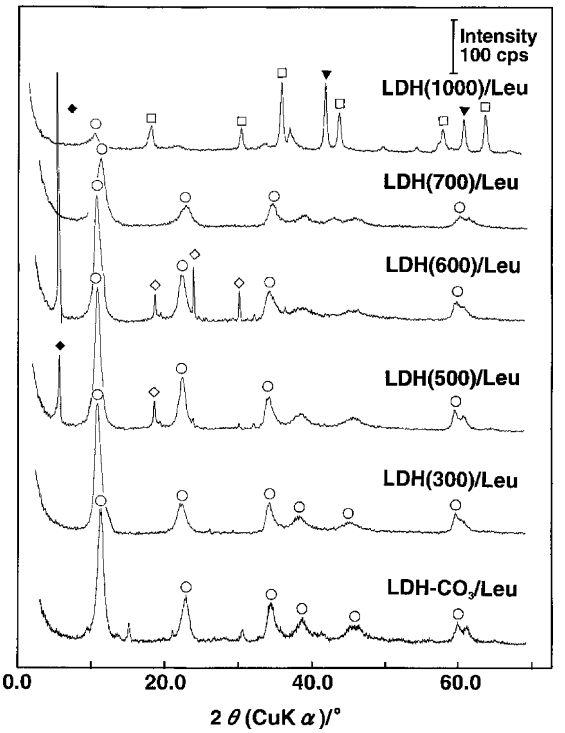


Fig. 1. XRD patterns of LDH(CO_3)/Leu by ion-exchange method, and LDH/Leu by reconstruction method. The numerical value in parenthesis is calcination temperature. Small diffraction peaks at $2\theta = 10$ and 15° in LDH(CO_3)/Leu are due to some impurities, which did not appear in LDH(*T*)/Leu at all. (○) LDH(CO_3), (▼) MgO, (□) $MgAl_2O_4$, (●) LDH/Leu.

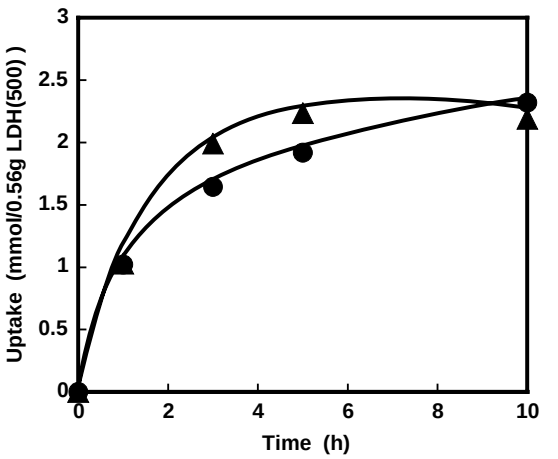


Fig. 2. Time dependence of the uptake of Phe into LDH(500) synthesized under N_2 gas (▲) and air (●).

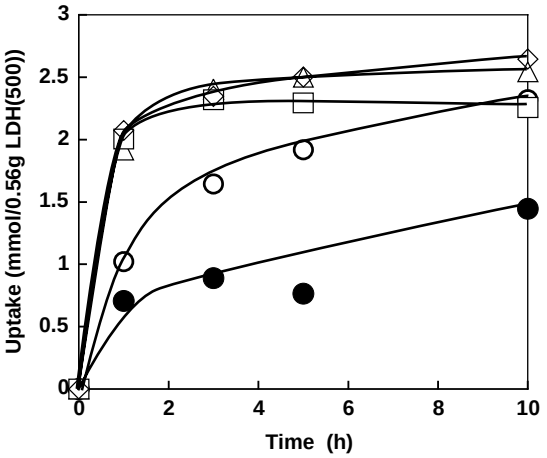


Fig. 3. Uptake of Phe into LDH(500) at different reaction temperatures. (●) 0 °C, (○) room temperature, (□) 40 °C, (△) 60 °C, (◇) 80 °C.

the maximum uptakes were almost the same at 24 h reaction for both cases. The presence of air did not affect the intercalation of Phe so much. Therefore, we performed the intercalation reaction without N_2 atmosphere from the practical point of view.

It is then examined the effects of reaction temperature and Phe concentration on the intercalation reaction. Fig. 3 shows the uptake of Phe into LDH(500) at different reaction temperatures. On increasing the reaction temperature, the rate of uptake became fast up to 40 °C, but there was no change above it. And the uptake of Phe saturated to 2.5 mmol. Although the rate of uptake was slow at room temperature, uptake reached to saturated value for 10 h. Therefore, it was enough to perform the reaction at room temperature for 10 h. The relation between uptake of Leu and initial concentration of Leu aqueous solution is shown in Fig. 4. The uptake increased linearly with initial concentration, and showed tiny plateau around 80 mmol l^{-1}. However, it again increased above this concentration. If the uptake of Leu obeys Langmuir adsorption isotherm, that is, monolayer exchange, the uptake will saturate at some concentration. Therefore, the uptake of Leu does not obey Langmuir type. In the case of adsorption in inhomogeneous surface, Freundlich equation as shown in Eq. (1) is normally applied (Moujahid et al., 2003).

$$\log W = \frac{\log C_{eq}}{n} + \log k, \quad (1)$$

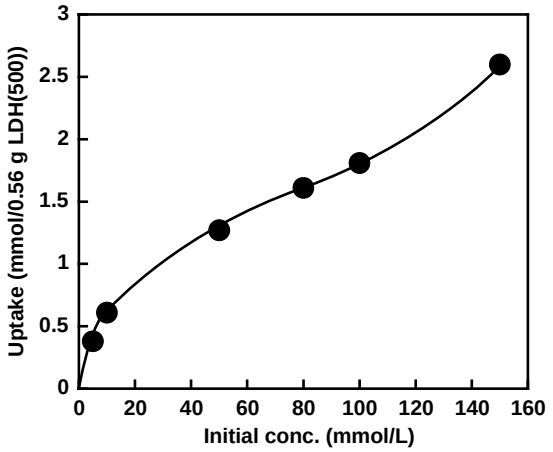


Fig. 4. Concentration dependence of uptake of Leu into LDH(500).

where W, C_{eq}, and n indicate uptake, equilibrium concentration of Leu, and the adsorption strength, respectively, and k is related to the adsorption amount. Application of Freundlich plot to this curve shows the straight line with $n = 2.6$, $k = 1.2$ as shown in Fig. 5. For most adsorbents n is 2–3 (Moujahid et al., 2003), confirming that LDH(500) is a good adsorbent of Leu. In the case of ion-exchange of other anions with Cl^- in LDH(Cl), the uptake obeys Langmuir equation (Nakayama et al., 2003). This is because the intercalated anion occupies one specific site in face of Al. In the case of amino acid, molecule exists as zwitterion in the reaction solution. Therefore, several intercalation sites might be expected. This will be discussed later.

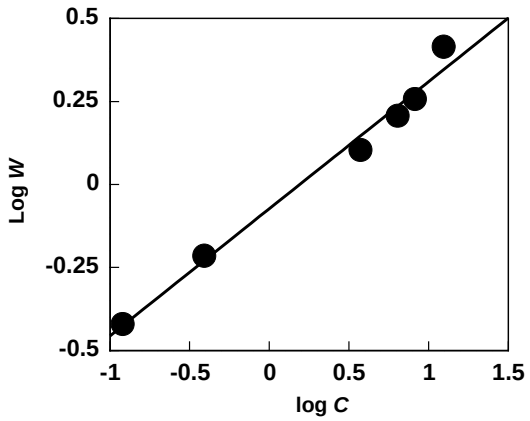


Fig. 5. Freundlich plot for Leu uptake into LDH(500).

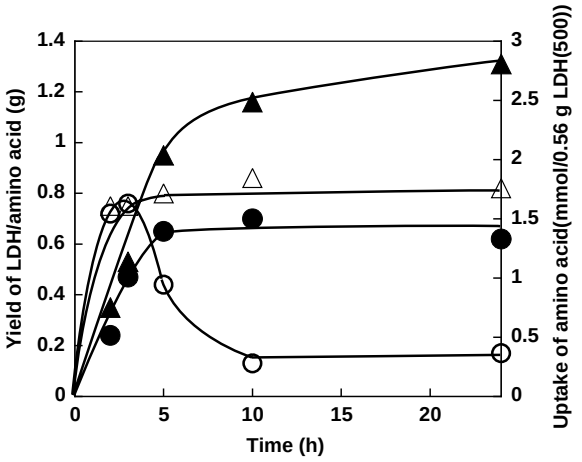


Fig. 6. Reaction time dependence of the yield of LDH(500)/Ala (○) and LDH(500)/Leu (△), and uptake of Ala (●) and Leu (▲) in the intercalation compounds.

For some amino acids, the dissolution of host LDH was observed during the intercalation reaction. Fig. 6 shows the reaction time dependence of the amount of LDH(500)/Ala (○) obtained by using 0.56 g LDH(500). Although the amount of the product was 0.8 g below 3 h, it decreased above 5 h, and eventually reduced to 0.15 g due to the dissolution of LDH. In some case, dissolution of LDH has been reported probably due to soluble chelete formation (Nakayama et al., 2003). Therefore, the recommended reaction time was 3 h for Ala. The interlayer distances and uptakes of LDH(500)/amino acid at the optimal reaction condition are summarized in Table 1. For Gly, Ala, Thr, Pro, Asn, Gln, and His, 3 h reaction was used, because the dissolution of LDH was observed for the longer reaction time. For Val, Leu, Ile, Phe, Trp, Ser, Cys, Met, Asp, and Glu, the longer reaction time was better to obtain the product with higher yield because of no dissolution (Fig. 6). All the amino acids except for Lys and Arg were easily intercalated into the calcinated LDH. Although acidic amino acids (Asp and Glu), Gln, and Asn were intercalated into the calcinated LDH with less than 1.3 mmol g^{-1}, the other amino acids were intercalated with the amounts of 1.7–3.0 mmol g^{-1}. These values are more than those by the coprecipitation method reported by Aisawa et al. (2001).

As shown in Table 1, there are two types of intercalation compounds. LDH(500)/Gly, for example,

Table 1
Interlayer distance and uptake of LDH/Amino acids at optimal conditions

Amino acid	d (Å)	Gallery height[a] (Å)	Uptake mmol per 0.56 g LDH(500)
Gly	7.8	3.0	2.5
Ala	7.8	3.0	2.6
Val	12.2	7.4	3.0
Leu	14.6	9.8	2.4
Ile	14.0	9.2	2.1
Phe	16.0	11.2	2.6
Trp	20.0	15.2	2.1
	26.0	21.2	
Ser	7.8	3.0	2.6
Thr	7.8	3.0	2.4
Cys	7.8	3.0	1.7
Met	15.3	10.5	2.6
Pro	7.8	3.0	2.7
Asn	7.8	3.0	1.3
Gln	7.8	3.0	0.9
Lys	–	–	–
Arg	–	–	–
His	17.5	12.7	2.6
Asp	7.8	3.0	1.3
Glu	7.8	3.0	1.3

(–): No intercalation compounds were obtained for Lys and Arg.
[a] $d - 4.8$ Å.

showed no expansion of the interlayer distance in spite of the uptake of 2.5 mmol Gly. Ala, Ser, Thr, Pro, Asn, Gln, Asp, and Glu also showed the same tendency. For the other amino acids, the intercalation induced the expansion of interlayer distance. For the former case, no expansion of the interlayer distance was observed in spite of the uptake of more than 0.9 mmol g^{-1} of LDH(500). This fact reminds that this adsorption might be surface adsorption. In order to reject this possibility, the surface area of the calcinated LDH(500) was measured by BET method to be 119 m^2 g^{-1}. This surface area is outer surface of the solid and not the interlayer space. If Gly was adsorbed on the outer surface of LDH(500) as monolayer, the adsorbed Gly was calculated to be 1.3×10^{-4} mmol g^{-1} of LDH(500), which is far below the measured uptake. Therefore, Gly must be intercalated into the interlayer space, although there was no expansion of the interlayer distance. Before intercalation, carbonate anion CO_3^{2-}, C_3 axis of which is perpendicular to the LDH layer, exists in the interlayer space with the gallery height of 0.3 nm. If the long axis of amino acid is parallel to the layer, it is not surprising

Table 2
Structural properties of LDH/amino acids

Amino acid	R	Size of R (Å)	*d* (Å)	Gallery height (Å)
Val	$-CH(CH_3)_2$	4.9	12.2	7.4
Ile	$-CH(CH_3)-CH_2-CH_3$	5.9	14.0	9.2
Norval	$-CH_2-CH_2-CH_3$	6.1	14.2	9.4
Leu	$-CH_2-CH(CH_3)_2$	6.1	14.6	9.8
Norleu	$-CH_2-CH_2-CH_2-CH_3$	7.4	15.8	11.0

for no expansion after the intercalation reaction. For the other amino acids, the intercalation induced the expansion of interlayer distance.

In order to investigate the structural aspect of the intercalation compounds in the latter case systematically, the intercalation of Norval and Norleu into LDH were also examined and summarized in Table 2. On increasing the long axis of amino acid molecule, the interlayer distance of LDH(500)/amino acid increased as well. In these intercalation compounds amino acids are expected to arrange with bilayer structure, because the expansion of the interlayer distance was larger than the long axis of amino acid molecule. This arrangement was recognized in LDH/Phe synthesized by the coprecipitation method (Aisawa et al., 2001) and obtained by the molecular dynamic simulation of LDH/Phe (Newman et al., 2002). Three amino acids with larger R groups showed the expansion of the interlayer distance. Therefore, hydrophobic interaction between R groups induced the bilayer structure accompanying the expansion of the interlayer distance.

In order to characterize the intercalation compound microscopically, solid-state ^{27}Al and ^{13}C NMR spectra were measured. Fig. 7 shows ^{27}Al MAS NMR spectra of LDH(500)/Leu and LDH(500)/Gly. ^{27}Al NMR spectra are very sensitive to a environment of Al

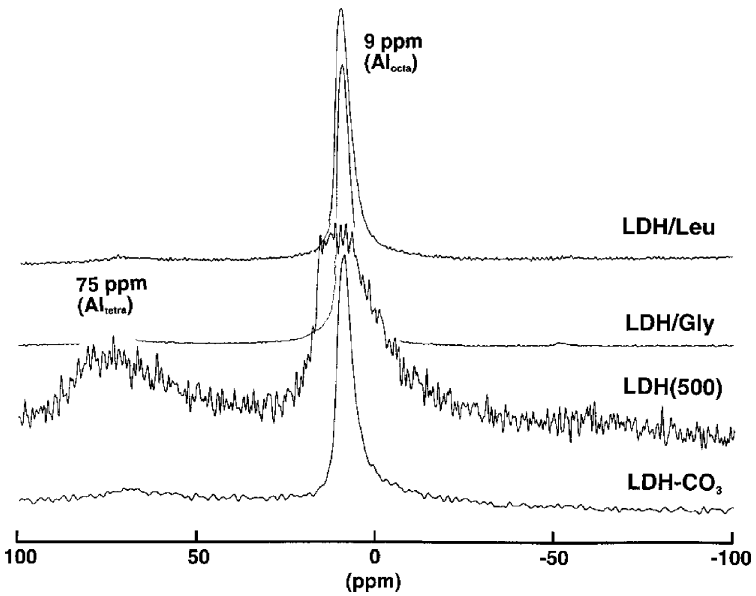
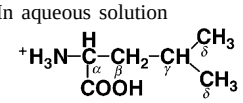
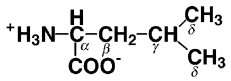
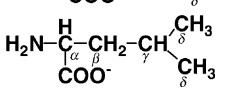


Fig. 7. ^{27}Al MAS NMR spectra of LDH(CO_3), LDH(500), LDH(500)/Gly, and LDH(500)/Leu.

atom (Rocha et al., 1999). The chemical shift value and line width reflect the coordination number and symmetry around Al atom. A sharp signal at 9 ppm shows six coordination with octahedral symmetry, and the narrow line width indicates the regular octahedral coordination with small distortion. This octahedral symmetry was deformed by the calcination at 500 °C to give another signal at around 75 ppm. However, the signal at 9 ppm did not change so much after the intercalation reaction of both Leu and Gly, suggesting no deformation of the LDH layer after the reconstruction reaction. The ionic state of amino acid, which depends on pH of an aqueous solution, can be estimated from the ^{13}C chemical shift value (Tran-Dinh et al., 1974; Horsley and Sternlicht, 1968). Table 3 shows the chemical shift values of LDH(500)/Leu together with those of Leu in aqueous solution at several pH. The chemical shift values of C_β and C=O carbons were very sensitive to pH, that is, ionic state of amino acid. The chemical shift values of LDH(500)/Leu are close to those of Leu^+ or zwitterion, although there are small discrepancy due to solid effect. Although Leu exists as anion in the reaction solution of pH 10, the intercalated Leu molecule might be zwitterion in the interlayer space. Narita et al. reported the intercalation of neutral molecule, such as monosaccharides (Aisawa and Narita, 2000), and we also reported that 1-hydroxyethylidene-1,1-diphosphonic acid, which is a analog of diphosphonate, could be intercalated into LDH as mono anion, although it existed as divalent anion in the reaction solution (Nakayama et al., 2003).

Table 3
^{13}C NMR chemical shift of Leu at different ionic state and LDH/Leu

	δ (ppm)				
	COOH	C_α	C_β	C_γ	C_δ
LDH/Leu	176	55	42	25	24
In aqueous solution					
	176.5	54.9	41.9	26.6	23.5
	178.1	56.0	42.4	26.8	23.5
	184.7	57.1	46.4	27.0	23.9

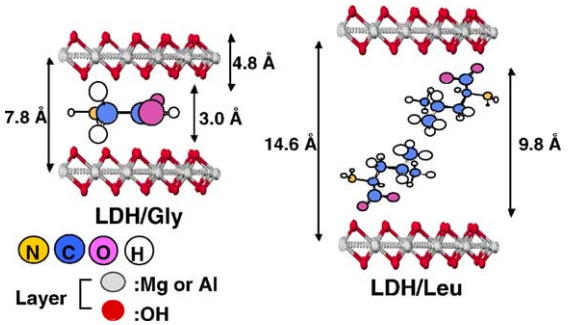


Fig. 8. Schematic structural models of LDH(500)/Gly and LDH(500)/Leu.

Because amino acid exits as zwitterion in the interlayer space of LDH, another anion, for example, OH^- or CO_3^{2-} must be intercalated at the same time from the point of electrical neutrality of LDH/amino acid, that is cointercalation. Therefore, amino acid would be interacted with the positive LDH layer not by Coulomb force but by hydrogen-bonding. This scheme is also confirmed by the deintercalation reaction.

Fig. 8 shows the schematic structural models of LDH(500)/Gly and LDH(500)/Leu. ^{27}Al MAS NMR spectra suggest that there is no deformation of LDH layer by the reconstruction reaction, and the distance between the layers just changed. In the case of amino acids with larger hydrophobic R group, the molecule arranges as bilayer structure in LDH as evidenced by XRD. In the case of the other amino acids, the long axis of amino acid molecule is parallel to the LDH layers.

3.2. Intercalation of aspartame and oligoglycines

For aiming the stabilization of enzyme and protein, two types of dipeptides were examined. Aspartame is an artificial sweetener with no calorie, and is composed of Asp and methyl ester of Phe. Because Phe has large hydrophobic R group, the expansion of the interlayer distance is expected for the intercalation of aspartame. On the other hand, glycylglycine (Gly-Gly) is expected to be no expansion of the interlayer distance because there was no expansion of the interlayer distance in the intercalation of Gly. Furthermore, Gly-Gly-Gly and Gly-Gly-Gly-Gly were examined as well.

The reaction time dependence of uptake of aspartame by the reconstruction method was shown

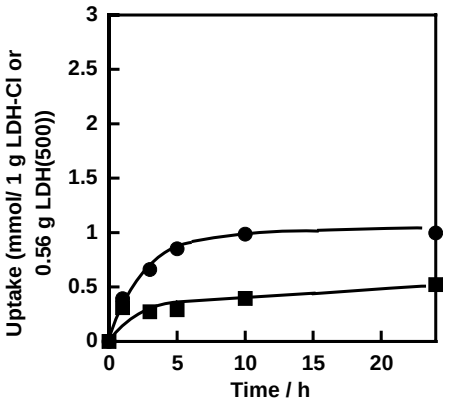


Fig. 9. Uptake of aspartame into LDH(Cl) (■) and LDH(500) (●) as determined by spectrophotometry. The intercalation reaction was carried out using 100 ml of 30 mmol l^{-1} aspartame aqueous solution.

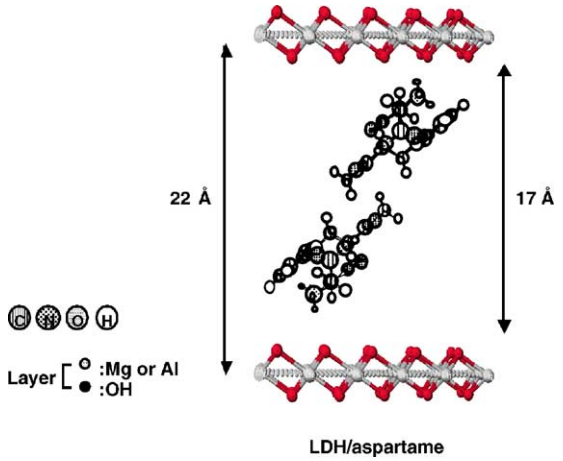


Fig. 10. Schematic structural model of LDH(500)/aspartame.

in Fig. 9. Because Asp is a acidic amino acid, ion-exchange method using LDH(Cl) was examined as well. Although aspartame was intercalated by both methods, the uptake by reconstruction method was 1.0 mmol, which is twice as good as that by ion-exchange method. As shown in Table 1, the uptakes of Asp and Phe were 1.3 and 2.6 mmol of 0.56 g of LDH(500), respectively. In the case of Asp, the dissolution of LDH and the lowering of uptake were observed with the reaction time. However, no dissolution of LDH was observed during the intercalation of aspartame, probably due to the presence of Phe. If the uptake was calculated based on the unit of amino acid, the uptake became 2.0 mmol of amino acid unit, which was comparable with Phe and the other amino acids. XRD pattern, and ^{27}Al MAS NMR spectrum of LDH(500)/aspartame showed the interlayer distance of 2.2 nm, and a sharp signal at 9 ppm, suggesting the bilayer structure of aspartame in the interlayer space as shown in Fig. 10. This bilayer structure is reasonable because Phe, which is a constituent amino acid of aspartame, also shows bilayer structure due to the presence of large hydrophobic phenyl group.

The intercalations of oligoglycines were examined using 100 ml of 50 mmol l^{-1} oligoglycine aqueous solution, and the data of intercalation compounds are summarized in Table 4. Similar to Gly, there was no change of the interlayer distance by the intercalation reaction, suggesting the parallel arrangement of the long axis of the molecules. As mentioned in LDH/amino acid, amino acids, which have absence of large hydrophobic group, have the parallel arrangement for the layer. This rule might also be applied to oligopeptides. The uptakes of all the oligoglycines examined were almost the same. Similar to aspartame, if the uptake was calculated based on the unit of amino acid, the uptakes become 2.4, 3.6, and 3.6 mmol for Gly-Gly, Gly-Gly-Gly, and Gly-Gly-Gly-Gly, respectively. In the case of oligopeptides, the hydrolysis of LDH during the reconstruction reaction must be considered, because LDH is a base catalyst. ^{13}C NMR spectrum was measured for Gly-Gly, as one example, and the data is summarized in Table 5. ^{13}C CP/MAS NMR of LDH(500)/Gly-Gly shows two C_α carbons of C-terminal and N-terminal glycines, indicating no hydrolysis of Gly-Gly intercalated and in solution. Although it is hard to estimate the ionic state of Gly-Gly intercalated, it might be zwitterion form from Table 5. The uptakes of oligoglycines are

Table 4
Interlayer distance, uptake and chemical shift value of LDH/oligoglycine

Oligoglycine	d (Å)	Uptake mmol/ 0.56 g LDH(500)	δ (^{27}Al) (ppm)
Gly	7.8	2.5	9
Gly-Gly	7.8	1.2	9
Gly-Gly-Gly	7.8	1.2	9
Gly-Gly-Gly-Gly	7.8	0.9	9

Table 5
^{13}C NMR data of LDH/Gly-Gly, Gly-Gly, and Gly

		δ (ppm)			
		C-terminal		N-terminal	
		$C00^-$	C_α	C=O	C_α
LDH/Gly-Gly		173.5	44.6	170.6	40.3
Gly-Gly in reaction sol.		179.4	46.1	176.9	45.8
Gly-Gly	Cationic	175.7	43.6	170.2	43.0
	Zwitterion	179.1	45.8	169.6	43.1
	Anionic	179.4	46.2	177.5	45.7
Gly		175.0	44.0		

comparable with amino acids, and the intercalation of larger peptides would be expected as well.

3.3. Deintercalation of amino acid and oligopeptide

In order to check the possibility as a DDS host, the deintercalation reaction of LDH/amino acid was examined. The pH of the reaction solution was 9–10. Fig. 11 shows the XRD pattern of LDH/Leu before and after the deintercalation reaction. The diffraction peak at 14.6 Å of LDH/Leu disappeared completely after the deintercalation by K_2CO_3 aqueous solution and H_2O. The elemental analysis and solid-state ^{13}C CP/MAS NMR spectra of the deintercalation compound also showed no evidence of Leu. Because the affinity of CO_3^{2-} to LDH is strong, it is natural to occur replacement of Leu with CO_3^{2-}. It was also confirmed by the ^{13}C NMR signal of CO_3^{2-}. ^{27}Al MAS NMR spectra showed no change by the deintercalation reaction. These facts suggest that LDH/Leu hybrid material is an intercalation compound, but not new unidentified compound. If the hybrid material is a new compound, the deintercalatin will not occur.

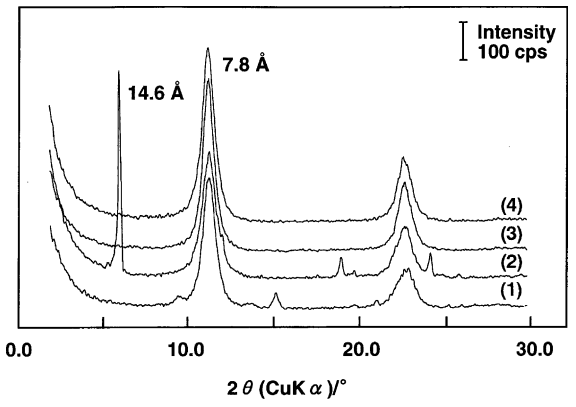


Fig. 11. XRD patterns before and after deintercalation reaction. (1) LDH(CO_3), (2) LDH(500)/Leu, (3) LDH/Leu after deintercalation in 25 mmol l^{-1} K_2CO_3 aqueous solution, and (4) LDH/Leu after deintercalation in H_2O.

However, it is surprising that the intercalated Leu was deintercalated by H_2O. Generally, the guest molecule, which is intercalated by ion-exchange method, cannot deintercalate in H_2O, because anionic guest molecule and positively charged LDH layer interacts with strong electrostatic interaction. As mentioned in the characterization of LDH/amino acid, most amino acids were intercalated as zwitterion. Therefore, amino acid molecule might be neutral in the interlayer space. Considering the electrical neutrality of the intercalation compound, this fact suggests that amino acid is intercalated with some other anions, for example, CO_3^{2-} and OH^-, that is cointercalation as mentioned before. If so, it is reasonable that amino acid was easily deintercalated in H_2O, because the interaction between positive LDH layer and zwitterions is hydrogen-bonding, which is not so strong. This cointercalation mechanism was also true for peptides.

Although easy release of amino acid in H_2O suggests the problem for the controlled release formulation, it shows that LDH/amino acid could be used as amino acid reservoir and adsorbent.

4. Conclusion

Amino acids- and peptides-intercalated LDH could be synthesized by the reconstruction reaction. The amounts of amino acids in the intercalation compound were around 2 mmol per 0.56 g of LDH(500). Although amino acids existed as monovalent anion in the reaction solution, they existed as zwitterions in the interlayer region of LDH.

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