High-Density Protein Loading on Hierarchically Porous Layered Double Hydroxide Composites with a Rational Mesostructure

Yasuaki Tokudome, Megu Fukui, Naoki Tarutani, Sari Nishimura, Vanessa Prevot, Claude Forano, Gowsihan Poologasundarampillai, Peter D. Lee, and Masahide Takahashi

ABSTRACT: Hierarchically porous biocompatible Mg–Al–Cl-type layered double hydroxide (LDH) composites containing aluminum hydroxide (Alhy) have been prepared using a phase-separation process. The sol–gel synthesis allows for the hierarchical pores of the LDH–Alhy composites to be tuned, leading to a high specific solid surface area per unit volume available for high-molecular-weight protein adsorptions. A linear relationship between the effective surface area, \( S_{\text{EFF}} \), and loading capacity of a model protein, bovine serum albumin (BSA), is established following successful encapsulation, and releasing large quantities of proteins was clearly demonstrated.

INTRODUCTION

Protein immobilization on solid surfaces is of relevance to a wide range of research areas, with potential applications in biotechnology and physiology. The activity of immobilized proteins is an important consideration, which affects inorganic/bio interfacial properties, such as antifouling and antibacterial properties and hemo-/biocompatibilities. Various solids have been studied as supports for proteins, including layered double hydroxides (LDHs), which are promising candidates as a result of their outstanding biocompatibility and an ability to limit denaturation of immobilized proteins. The active conformation of proteins is retained on two-dimensionally flat and highly hydrophilic surfaces of LDHs, to avoid denaturation, which otherwise takes place on curved inorganic surfaces. As a result, heme proteins, which usually denature on inorganic solids, can be immobilized on LDH surfaces without losing their inherent activity. Immobilized heme proteins are currently used as bioelectrodes with high sensitivity.

Synthesizing LDHs with meso-/macropores is highly promising to achieve high capacity loading of proteins. The rational design of the porous structure in the nanometer to submicrometer scale is especially important because micropores (<2 nm) and relatively small mesopores that typically adsorb ion/small molecules cannot accommodate large protein molecules (~tens of nanometers in size). The surface area accessible by proteins (defined here as effective surface area, \( S_{\text{EFF}} \)) strongly depends upon the pore diameter, \( D_p \), of porous LDHs. To date, LDHs of micrometer and sub-micrometer scale loading. Recently, we have reported the preparation of monolithic LDHs with hierarchical pores. The hierarchical pores of macro (1 \( \mu \)m) and meso (8 nm) formed
spontaneously via a facile sol–gel reaction. It was also reported that target oxyanions (CrO$_4^{2-}$, SO$_4^{2-}$, MoO$_4^{2-}$, etc.) and small molecules diffuse rapidly through the macropores and adsorb on a large surface derived from the mesopores.\textsuperscript{16} However, the development of LDHs with tens of nanometer pores, which are optimized to maximize \( S_{\text{EFF}} \) through structural hierarchy, is still needed to exploit the applications of biocompatible solid supports with a high capacity for protein loading.

We prepare here biocompatible composites of LDH and aluminum hydroxide (Alhy) with two levels of hierarchical tunable pores in the range of tens of nanometers and a few micrometers. Dependence of the mean mesopore diameter, \( D_{\text{pm}} \), upon the synthesis parameters was investigated to tune the hierarchical porosity of the LDH–Alhy. Then, the adsorption of a large protein, bovine serum albumin (BSA), on the LDH–Alhy surfaces of various \( S_{\text{EFF}} \) was conducted to establish a relationship between \( S_{\text{EFF}} \) and loading capacity (Figure 1a). \( D_{\text{pm}} \) could be tuned from 12 to 53 nm, leading to a wide range of \( S_{\text{EFF}} \) for adsorption of BSA molecules. Particular interest was focused on aerogels obtained via supercritical drying,\textsuperscript{17} on which considerable adsorption of the large protein molecules was achieved as a result of maximized \( S_{\text{EFF}} \). Finally, the capability of the LDH–Alhy to release the immobilized protein in HPO$_4^{2-}$ and Cl$^-$ aqueous solutions was investigated (Figure 1b). The encapsulation as well as the release of BSA was attempted by inducing the shrinkage of the porous matrix after BSA loading (Figure 1c). The results demonstrated here provide key quantitative insights into protein loading on hydroxide-based biocompatible materials that display 14 times higher loading capacity than referential LDH materials.

**EXPERIMENTAL SECTION**

**Chemical.** Aluminum chloride hexahydrate (AlCl$_3$·6H$_2$O, 98%) and magnesium chloride hexahydrate (MgCl$_2$·6H$_2$O, 98%) were used as inorganic sources. A mixture of ultrapure water and ethanol (EtOH, 99.5%) was used as a solvent. (±)-1-Propylene oxide (PO, >99%) and isopropyl alcohol (IPA, 99.7%) were employed as a basic reagent and a liquid for sample washing, respectively. Poly(ethylene oxide) [PEO; viscosity average molecular weight \( \text{Mv} = 1 \times 10^6 \)] was used as an organic additive. Dipotassium hydrogen phosphate (K$_2$HPO$_4$ >99%), sodium chloride (NaCl, >99%), and hydrochloric acid (HCl, 36.0 wt %) were used to trigger desorption of BSA. PO, PEO, and BSA were purchased from Sigma-Aldrich Co., and all other reagents were from Wako Pure Chemicals Industries, Ltd. All chemicals were used as received. For comparison, reference LDH (Ref-LDH) was prepared by a standard pH constant co-precipitation approach\textsuperscript{18} with a chemical composition of [Mg$_{0.68}$Al$_{0.32}$(OH)$_2$Cl$_{0.21}$(CO$_3$)$_{0.06}$]. A commercial LDH [Com-LDH, Mg$_{0.88}$Al$_{0.67}$(OH)$_2$(CO$_3$)$_{0.06}$] was purchased and used for further comparison.

**Preparation of LDH–Alhy Aerogels and Aerogels.** Typically, AlCl$_3$·6H$_2$O (1.58 g, 6.55 mmol), MgCl$_2$·6H$_2$O (1.06 g, 5.23 mmol), and various amounts \( W_{\text{PEO}} \) of PEO were dissolved in a mixture of water/ethanol (4.00 mL/3.00 mL). PO (1.82 mL, 26.2 mmol) was added to this solution, maintained at 25 °C and stirred for 1 min, to yield a homogeneous sol. The sol was transferred to a polystyrene container, sealed, and kept at 40 °C. After 24 h, an opaque gel, thus obtained, was soaked in IPA for 1 h to exchange the water/ethanol solvent that remained inside pores with IPA. This process was repeated at least 8 times with fresh IPA. Then, the gel was solvothermally treated in IPA at 180 °C for 24 h, followed by either ambient or supercritical drying. The ambient drying was performed at 40 and 120 °C in an oven to yield aerogels, named as “X-LDH-40” and “X-LDH-120”, respectively. Supercritical drying was conducted with supercritical CO$_2$ (80 °C and 14.0 MPa), yielding aerogels, which are labeled as “A-LDH” in the following.

**Structural Characterization.** Fine structures of the samples were observed by field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi, Japan). Crystal phases of the obtained samples were identified by X-ray diffraction (XRD, MultiFlex, Rigaku, Japan) using Cu K$\alpha$ radiation (\( \lambda = 0.154 \) nm). Micro–mesoporous characters of the samples were investigated by a N$_2$ adsorption–desorption apparatus (BELSORP-mini II, Bel Japan, Inc., Japan). Prior to the measurement, the samples (A-LDH, X-LDH, Com-LDH, and Ref-LDH) were subjected to quick heat treatment to remove adsorbed water at 500 °C and then further outgassed under vacuum at 200 °C. The pore size distribution was calculated from the adsorption branch of the isotherm by the Barrett–Joyner–Halenda (BJH) method. The mean pore diameter, \( D_{\text{pm}} \), and total pore volume, \( V_p \) (cumulative pore volume calculated from pores with \( D_p < 183 \) nm), were estimated from the distribution curves. The BJH method was also applied to assess partial
specific surface area $S_{(>x)}$ derived from pores larger than a cutoff value. For example, $S_{(>5 \text{ nm})}$ corresponds to a specific surface area obtained by integrating surfaces of pores with $D_P > 5 \text{ nm}$. The Brunauer–Emmett–Teller (BET) method was also applied to estimate the specific surface area, $S_{\text{BET}}$. Synchrotron X-ray micro-computed tomography ($\mu$-CT) was employed to non-destructively obtain three-dimensional (3D) images of the drying process of the monoliths. A high-resolution synchrotron-based X-ray tomographic image was obtained from the Diamond-Manchester branchline I13-2 at Diamond Light Source. A polychromatic filtered parallel-beam setup was used with a $0.81 \mu\text{m}$ effective pixel size and $\sim2 \mu\text{m}$ spatial resolution. Over the $180^\circ$ rotation, 3600 projections were collected at 0.05 s exposure time and tomographically reconstructed into a 3D volume using software developed at Diamond Light Source. Visualization package Avizo was used to produce the 3D images and quantify shrinkage. The gel samples for the $\mu$-CT measurements were prepared from $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2.19 g), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.553 g), $W_{\text{PEO}} = 0.03 \text{ g}$, water (4.00 mL), ethanol (3.00 mL), and PO (2.27 mL).

**BSA Sorption Test.** Each of X-LDH (25 mg/mL), A-LDH (1.5 mg/mL), Ref-LDH (7.5 mg/mL), and Com-LDH [10 mg/mL, Mg$_6$Al$_2$(OH)$_{16}$CO$_3$·4H$_2$O, Wako Pure Chemicals Industries, Ltd.] was dispersed in aqueous BSA solutions of various concentrations (1.0–3.5 mg/mL) (the supporting note is in the Supporting Information). The mixtures were placed at 25 °C for 72 h. Supernatants of respective mixtures were collected through a membrane filter (0.45 μm) and analyzed by ultraviolet–visible (UV–vis) spectroscopy (V-670 spectrophotometer, JASCO Corp.). The concentration of BSA was estimated by the Beer–Lambert law using a peak intensity at 277.4 nm. The amount of adsorbed BSA was calculated from eq 1

$$C_s = \frac{(C_i - C_{eq})V}{m}$$

where $C_s$ (mg/g) is the amount of BSA adsorbed by LDH-Alhy composites, $C_i$ (mg/mL) and $C_{eq}$ (mg/mL) are initial and equilibrium concentrations of BSA in the solution, respectively, $V$ (mL) is the volume of the solution, and $m$ (g) is the mass of LDH. The Freundlich equation (eq 2) was used as the isotherm model for BSA adsorption on the sample solids

$$\log C_s = \log K_f + \frac{1}{n_f} \log C_{eq}$$

where $K_f$ (mg/g) is the Freundlich constant and $n_f$ is the adsorption intensity. The BSA adsorption tests were conducted on LDH–Alhy composites prepared at $W_{\text{PEO}} = 0, 0.01, 0.02, 0.03$, and 0.04. To study BSA desorption, 30 mg of the sample, which had previously adsorbed
BSA in the solution of the BSA concentration of 2.5 mg/mL (25 °C for 72 h) was immersed in 0.1 M NaCl and 0.1 M KHP04 aqueous (25 °C for 72 h), respectively. The amounts of BSA molecules desorbed from A-LDH ($W_{PEO}$ = 0.03 g) and Com-LDH were analyzed by the UV–vis technique. Some A-LDHs were dried after BSA adsorption (before the desorption test), and the effect of shrinkage on desorption was investigated. The reproducibility of adsorption isotherms was assessed on Com-LDH and X-LDH-40 (details in the Supporting Information).

### RESULTS AND DISCUSSION

#### Synthesis of Hierarchically Porous LDH–Alhy with Tunable Porosities

The hierarchically porous LDH–Alhy composites were prepared via hydrolysis and condensation reactions of metal salts by alkalinization in the presence of PO according to our previously published method.\textsuperscript{15} A nucleophilic attack of Cl\textsuperscript{−} induces the ring-opening reaction of PO, and subsequent alkalinization increases pH of the solution to precipitate LDHs.\textsuperscript{22,23} This reaction process yields nanosized hydroxide particles (crystals) and leads to homogeneous gelation in 15 min as a result of a high degree of supersaturation generated in the reaction solution. The gelling solution phase separates\textsuperscript{24} into a LDH–Alhy-rich solid phase and a PEO-rich fluid phase. Evaporative removal of the fluid phase leaves macropores (in the micrometer range). Simultaneously, the mesopores (in the nanometer range) were formed within the gel skeleton (Figure 1a). The mesopore size, $D_{pm}$ and mesopore volume, $V_p$, which expectedly influence $S_{PEG}$ and the adsorption capacity of proteins, varied as a function of $W_{PEO}$ (the amount of PEO additive) and drying conditions.

Figure 2 shows XRD patterns and scanning electron microscopy (SEM) images of the different LDH–Alhy composites and Ref-LDH. The composites and Ref-LDH are ascribed to hydrotalcite-type LDH with typical powder X-ray diffraction (PXRD) patterns of a $\gamma\text{m}$ hexagonal lattice.\textsuperscript{25} Crystallite sizes estimated from Scherrer’s equation using the (003) diffraction peak are 24 nm for Ref-LDH, 7.2 nm for X-LDH-40, 7.1 nm for X-LDH-120, and 6.8 nm for A-LDH. These values provide evidence that the crystallite sizes of X-LDH and A-LDH are comparable and far smaller than Ref-LDH used here as the reference, considered as a poorly crystallized material. Chemical analysis revealed that the LDH–Alhy composites have the composition of [Mg$_{6.66}$Al$_{0.33}$]$_{2+}$CO$_{3.33}$−$\frac{1}{2}$$\cdot$H$_2$O·2.0Al(OH)$_3$. Alhy, which forms porous networks together with LDH crystals, displays a hydroxylated surface that is biocompatible with proteins, as confirmed by its use as an adjuvant in some vaccines.\textsuperscript{26} As a result, the LDH–Alhy composites offer an extensive solid surface used for protein loading.

The LDH–Alhy composites produced here and Ref-LDH possess very different microstructures (Figure 2). The morphology of Ref-LDH is aggregates of LDH plates with the size of 1–4 μm. On the other hand, LDH–Alhy composites possess hierarchically porous structures with a monolithic form. As well as interconnected macropores with the pore diameter of 1–2 μm, mesopores are confirmed in the gel skeletons (higher magnification images in Figure 2). The difference of mesostructures is clearly supported by $N_2$ sorption measurements (Table 1). Large BET surface areas exceeding 400 m$^2$/g are derived from the mesoporosity, which are formed as interstices of constituent nanoparticles. The BJH pore size distributions of Ref-LDH, Com-LDH, and LDH–Alhy composites are plotted in Figures S1 and S2 of the Supporting Information. Com-LDH and Ref-LDH have negligible mesopores, whereas LDH–Alhy composites possess a considerable pore volume of 0.94–4.27 cm$^3$/g, originated from pores in the range of $D_p < 150$ nm. PEO changes the assembly of LDH and Alhy particles, and $D_{pm}$ decreases with increasing $W_{PEO}$ (Table 1). The formation of smaller mesopores with increasing $W_{PEO}$ is caused by a decrease of the solvent, which is to be mesopores after drying in the LDH–Alhy-rich solid phase, leading to more packed LDH and Alhy crystallites.\textsuperscript{27} The characteristics of mesopores also depends upon the drying conditions. A-LDHs possess the largest $D_{pm}$ and $V_p$ among the three sets of LDH–Alhy composites because the shrinkage is minimized by applying the supercritical drying.\textsuperscript{17} To obtain better insight on the shrinkage upon the drying process of the monolithic LDHs, X-ray $\mu$-CT was performed while the gels were left to dry at 300 K (Figure 3). Figure 3 shows 3D images of a small volume of the same gel before and after drying. It shows that shrinkage was isotropic, leading to a volume shrinkage of 85% and a linear shrinkage of 46%. The relatively large $D_{pm}$ and $V_p$ of X-LDH-120 compared to X-LDH-40 is due to a smaller degree of shrinkage upon drying. The faster drying at 120 °C forms cracks in the monolithic specimen and releases stress generated at the drying front, retarding isotropic shrinkage and leading to larger mesopores.\textsuperscript{28} It should be emphasized again that X-LDH-40, X-LDH-120, and A-LDHs possess the identical chemical composition and crystallinity, and differences among these samples are only porosity in nanometer and micrometer scales. In summary, $D_{pm}$ of LDH–Alhy composites was successfully tuned between 12 and 53 nm by the $W_{PEO}$ value and drying conditions.

#### Effect of the Pore Structure on Protein Adsorption

The LDH–Alhy composites with various $D_{pm}$ and $V_p$ were assessed as bio-supports with a high capacity for protein loading. As an adsorbate, BSA was used, which is a large multi-domain protein with a hydrodynamic radius of 3.6 nm and 3D size of $5 \times 7 \times 7$ nm$^3$;\textsuperscript{29} Serum albumin, a major soluble constituent of the plasma proteins, has many physiological functions.\textsuperscript{30} Moreover, BSA has been used as a model protein to investigate reactions of physiological disorders, such as diabetes,\textsuperscript{31} and its sustained release,\textsuperscript{32} immobilization, and bioprobes\textsuperscript{33} are of interest. Aluminum hydroxide and LDH have been individually used in the form of crystalline platelets as adsorbents for BSA molecules.\textsuperscript{34–36} Preliminary results (not shown) of BSA adsorption using previously reported hierarchically porous LDHs\textsuperscript{37} (prepared by ambient drying without the solvothermal treatment) lead to a negligible amount of protein...
adsorption, where the mesopore size was too small to accommodate protein molecules and only macropores were available for protein adsorption; the surface area derived from macropores is less than 10 m$^2$ g$^{-1}$.

Figure 4a represents adsorption isotherms of BSA on X-LDH-40 at different W$_{PEO}$. At W$_{PEO} = 0$, 0.01, and 0.02 g, the adsorption of BSA was negligible. LDH–Alhy composites prepared at W$_{PEO} = 0$ and 0.01 g do not possess any macropores, limiting BSA adsorption to the external surface, where BSA adsorbed on the surface prevents further diffusion of protein through the mesopores into the bulk of the material. On the other hand, LDH–Alhy with a hierarchically porous structure (W$_{PEO} = 0.03–0.04$ g and macropore size = 1–2 μm) shows much higher BSA loading as a result of the presence of co-continuous macropore channels for BSA molecules to diffuse through to the majority of the available surface. Corresponding adsorption isotherms of X-LDH-120 and A-LDH are plotted in panels b and c of Figure 4, respectively. As a result of the lack of realistic adsorption models for fitting the interactions between solid surfaces and proteins, many studies have reported the use of the Freundlich model as an approximative tool to evaluate and compare various solid/protein systems. Freundlich adsorption isotherms for X-LDH-40, X-LDH-120, and A-LDH are represented in Figure S3 of the Supporting Information. The plots can be linearly fitted by eq 2, except for the case of X-LDH-40 at W$_{PEO} = 0.02$ g, whose microstructure is highly inhomogeneous because of structure deformation during drying. The $K_F$ value increases in the order of X-LDH-40 < X-LDH-120 < A-LDH, as summarized in Table 2. The Freundlich constant, $K_F$, of A-LDH prepared with W$_{PEO}$ = 0.02 g is 996 mg g$^{-1}$. The value is 14 times that of Ref-LDH (70 mg g$^{-1}$), confirming that A-LDH composites prepared in the present study exhibit excellent adsorption properties compared to X-LDH, Ref-LDH, and Com-LDH. Clearly, the porous structure is the key factor that accounts for BSA loading, while other influences, slight pH change during adsorption and difference of hydroxylated surfaces, are negligible (Tables S1 and S2 of the Supporting Information). Indeed, while the surface of Ref-LDH with a higher ζ potential (+42 mV) should promote higher BSA adsorption than the LDH–Alhy composite (+37 mV for X-LDH-40), the reverse is observed.

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**Table 2. Summary of Freundlich Fitting of BSA Adsorption on Solid Surfaces**

<table>
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<tr>
<th>entry</th>
<th>W$_{PEO}$</th>
<th>$K_F$ (mg g$^{-1}$)</th>
<th>1/$n_F$</th>
<th>$R^2$</th>
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<tr>
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<td>0.28</td>
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<tr>
<td></td>
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Figure 3. 3D and two-dimensional (2D) X-ray μ-CT images of the LDH–Alhy composites before (wet gel) and after (xerogel) drying at 300 K. The wet gel sample for μ-CT was prepared at Mg/Al = 0.3 and W$_{PEO}$ = 0.03 g. Isotropic shrinkage took place during the drying, leading to the volume shrinkage of 85% and the linear shrinkage of 46%. Images were reconstructed from the same part of the monolithic sample. The yellow dotted lines in 3D images represent 2D planes shown as 2D images.

Figure 4. BSA adsorption isotherms on (a) X-LDH-40, (b) X-LDH-120, and (c) A-LDH at 25 °C. Adsorption isotherms of BSA on A-LDH prepared at W$_{PEO}$ = 0 and 0.01 g (without macropores) are not reproducible.
BSA has an isoelectric point of pI 4.7\textsuperscript{39} and is negatively charged in the present adsorption condition (pH ~ 7).

Figure 5 shows plots of $K_T$ values of LDH–Alhy composites against $S_{(x \geq 5 \text{ nm})}$ that is a specific surface area obtained by integrating surface areas derived from pores of $D_p > x \text{ nm}$; for example, $S_{(5 \text{ nm})}$ is a sum of surface areas derived from pores with a diameter of $D_p > 5 \text{ nm}$. The LDH–Alhy composites prepared in the present study have tunable pore characteristics, and $S_{(x \geq 5 \text{ nm})}$ was controllable to a large extent. Figure 5 shows $K_T$ values against $S_{(x \geq 5 \text{ nm})}$ for $x = 5, 10, 15$, and $20 \text{ nm}$ pore size. $K_T$ and $S_{(x \geq 5 \text{ nm})}$ do not have correlation because relatively small mesopores cannot accommodate BSA. The surface area, which is derived from the mesopores whose sizes are insufficient for BSA adsorption, contributes less to $S_{(x \geq 5 \text{ nm})}$ as the $x$ value becomes larger. As a result, linear least squares fitting gives a better correlation coefficient $r$ with an increasing $x$. At $x = 15$ and $20 \text{ nm}$, $r$ was estimated as 0.77 and 0.92, respectively. These results reveal that $D_p$ of ca. 20 nm is the threshold, which allows for the accommodation and adsorption of BSA molecules on the entire surfaces of mesopores. Although the pores of 10–20 nm are apparently larger than the size of BSA molecules ($5 \times 7 \times 7 \text{ nm}^3$), adsorption on the surface of these relatively small mesopores does not take place efficiently because the entrance of mesopores is blocked by the first few BSA molecules adsorbing there. This effect was indeed observed for X-LDH-40 ($W_{rad} = 0–0.02$), in which mesopores ($D_{meso} < 15 \text{ nm}$) in the absence of macropores exhibited negligible adsorption (Figure 4a). A similar size effect of mesopores on BSA adsorption was qualitatively reported on mesoporous silica, where SBA-15 with the pore diameter of 24 nm showed much better BSA adsorption than those with 3.8 and 7.7 nm in diameter.\textsuperscript{40} The present results give very systematic and quantitative evidence for a threshold in the pore diameter for effective adsorption of BSA molecules to mesoporous materials. The approach demonstrated here will be a promising platform to maximize $S_{EFF}$ for respective proteins with different molecular sizes.

**Ability for Encapsulation and Release of Immobilized Proteins.** The capability of encapsulating immobilized protein and their sustained release are also an important feature for bioadsorbents. A previous study reported that BSA desorption from Zn–Al LDH took place by the addition of competitive anionic adsorbates.\textsuperscript{41} Herein, HPO$_4^{2-}$ and Cl$^-$ ions were used as competitive ions to release pre-adsorbed BSA molecules. Table 3 summarizes the results of protein desorption from A-

![Figure 5. $K_T$ versus $S_{(x \geq 5 \text{ nm})}$ of LDH–Alhy composites. $K_T$ values of LDH–Alhy composites listed in Table 2 are plotted. $S_{(x \geq 5 \text{ nm})}$ = specific surface areas derived from the pores larger than $x \text{ nm}$. The results of linear least squares fitting are depicted with the plots. $r = correlation coefficient.](image)

LDH and Com-LDH. A total of 52% of BSA desorbed in 72 h from A-LDH in the case of using HPO$_4^{2-}$ as a competitive anion, which is comparable to that from Com-LDH (49%). This result confirms that the protein desorption and adsorption take place with a large capacity for the present LDH–Alhy composites. Cl$^-$ ions did not induce the desorption of BSA for both Com-LDH and A-LDH, which is due to the lower affinity of Cl$^-$ and LDH surface and smaller negative charge compared to HPO$_4^{2-}$. While the LDH–Alhy composites (X-LDH and A-LDH) have a weight ratio of LDH/Alhy = 1:1, the desorption percentage by Cl$^-$ of these composites is almost the same as pure LDH (~2%) (Table 3). A simple integration of respective surfaces of LDH and Al(OH)$_3$ cannot explain the low BSA desorption from the A-LDH composite observed with Cl$^-$ as the counterion, because BSA desorption easily occurs on Alhy (50–60%) using Cl$^-$ as a competitive ion. The unique surface texture of A-LDH, the nanosporic surface composed of Alhy and LDH nanoparticles, would result in the peculiar restricted desorption, although further evidence is required. BSA molecules were also entrapped in the matrix of the composite by first immobilizing BSA within A-LDH, which was then dried to trap the protein within as the gel shrunk. As summarized in Table 3 and Figure 6, the desorption of BSA molecules by HPO$_4^{2-}$ from dried samples was <23%, which is less than half that of the non-dried sample of its original dimension (52%). These results demonstrate the possibilities of encapsulation and extended release of various protein molecules with a high capacity based on tunable hierarchically porous LDH. Combining LDH materials with various selectivities toward different molecules/ions will further open up biocompatible separation and purification required for biomedical applications.\textsuperscript{42}

**CONCLUSION**

The preparation of biocompatible LDH–Alhy composites with an optimized $S_{EFF}$ for large protein molecules is presented. $S_{EFF}$ could be altered by the synthesis parameters, such as the amount of polymer additive and the drying conditions used. The capacity for protein loading was investigated with BSA as a model protein, which revealed the existence of a threshold on...
the smaller critical mesopore size required to accommodate BSA molecules to be ca. $D_p = 20$ nm. Especially, the LDH–Alhy aerogel ($W_{\text{FO}} = 0.02$ g) with a hierarchically porous structure and median mesopore size of 53.2 nm exhibited a remarkably high protein loading ($K_i = 996$ mg g$^{-1}$) when compared to Ref-LDH and Com-LDH standards. The BSA molecules pre-adsorbed on the composite were released by competing ion, BSA release was retarded to the extent comparable to pure LDH, presumably because of the unique surface texture, nanomosaic of LDH and Alhy. The tunable mesostructures in the range of nanometers to tens of nanometers are applicable to molecular sieving and purification of protein solutions. Further investigation based on this platform would open up many possibilities of using these hierarchically porous LDH–Alhy composites in various applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b01925.

N$_2$ sorption isotherms and BJH pore size distributions of LDH–Alhy and referential samples, linearized form of the adsorption isotherm according to the Freundlich model, influences of the heat drying temperature, pH, and sample mass on BSA adsorption, reproducibility of analysis based on the Freundlich equation, and influence of thermal treatment prior to N$_2$ adsorption measurements (PDF)

AUTHOR INFORMATION

Corresponding Author
*E-mail: tokudome@photomater.com.

Notes
The authors declare no competing financial interest.

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