Study on the intercalation and interlayer oxidation transformation of L-cysteine in a confined region of layered double hydroxides

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Two kinds of amino acids (L-cysteine and L-cystine, represented as L-CySH and L-Cys respectively) have been intercalated into MgAl layered double hydroxides by coprecipitation. The structure and composition of the intercalated materials have been characterized by X-ray diffraction (XRD) and elemental analysis. Furthermore, the oxidation of L-CySH intercalated LDHs by, respectively, hydrogen peroxide and bromine has been studied. Based on the FT-Raman, FT-IR, and 13C MAS NMR spectra data, it has been found that H2O2 oxidized the interlayer L-CySH to cystine, while the oxidation product of intercalated L-CySH by Br2 was cysteic acid regardless of the bromine/cysteine ratio, which is different from the bulk reaction. Therefore, this layered material may have prospective application as the basis of a novel “molecular reactor” for confined chemical reactions.

Introduction

Layered double hydroxides (LDHs), also known as hydro-talcite-like compounds, are ionic lamellar compounds that consist of positively charged hydroxide sheets and interlayers filled with anions and water molecules. The most common group of LDHs can be represented by the general formula M(II)1−xM(III)x(OH)yA3n/mH2O, where M2+ and M3+ are di- and trivalent metal cations occupying octahedral positions within the host layers of hydroxide sheets, and A”− is an interlayer anion that compensates for the charge on the layers. Positive charge on the host layers arises from isomorphous replacement of a fraction of the divalent cations with trivalent cations.

These layered solids based on the alternation of inorganic and organic layers have received considerable attention due to their potential technological applications in the fields of catalysis,2−5 electrodes in potential memory,6 optical materials,7,8 antacids,9 separators10,11 and synthesis material12,13 for organic–inorganic nanocomposites. The attractive feature of such materials is that they serve as a template for the formation of supramolecular structures. Many species can be assembled by reaction of guest species in the LDH matrices,2,14 and intercalation of biomolecules or pharmaceutical agents into LDHs has become of interest in recent years.14−16 The intercalation of various amino acids17 as well as nucleoside monophosphates and DNA18 has been reported.

The sulfhydryl and disulfide groups, which are present in amino acids, peptides, proteins and enzymes, are known to play important biochemical and physiological roles.19 Cysteine is a non-essential amino acid which can be synthesized in the human body through the metabolism of methionine.20 As a result of containing sulfur, cysteine can bond in a special way to maintain protein structure in the body. Moreover, as an antioxidant, cysteine is able to prevent the formation and oppose the actions of reactive oxygen species which are generated in vivo and cause damage to DNA, lipids, and proteins.21,22 It is assumed that one of its most important roles is in forming and breaking of S–S bonds.23 J. Darkwa et al.24,25 studied the reaction dynamics, stoichiometries and mechanism of the oxidation of cysteine by several well known oxidants. It has been found that the oxidation of cysteine is facile, rapid and the ratio of oxidant/reductant has a remarkable influence on oxidation products.

However, to the best of our knowledge, there has been no report on the investigation of the oxidation of cysteine in a restricted region. In the present work, L-CySH and L-Cys have been intercalated into LDHs by the coprecipitation method. Furthermore, the oxidation of intercalated L-CySH has been studied by using two common oxidants, H2O2 and Br2, respectively. It was found that the oxidation product was influenced by the confined environment of the LDHs nanoscale gallery, and differs from the bulk reaction. The host layers perform as a novel “molecular reactor” to impose restricted geometry on the interlayer guests leading to enhanced control of stereochemistry, rate of reaction, and product distribution. Therefore, the study of the oxidation transformation of cysteine in confined regions between LDH sheets opens new opportunities for further research of molecular reactions on the nanoscale of inorganic hybrids and for their use in catalytic applications.

Experimental

Materials

The following analytical grade chemicals were purchased from Beijing Chemical Plant Limited and used without further purification: NaOH, Mg(NO3)2·6H2O, Al(NO3)3·9H2O, H2O2 (30 wt.% solution in water), Br2. L-cystine and L-cysteine were purchased in analytical reagent grade quality from Sigma.
Preparation of L-CySH and L-Cys LDHs

L-CySH and L-Cys LDHs were prepared by the method of coprecipitation,26 which is the most common preparative technology of LDHs. In the coprecipitation method, the M2+ and M3+ hydroxide layers are nucleated and grown from an aqueous solution containing the anion that is to be incorporated into the LDHs.27 The matched molar ratio of Mg2+/Al3+/OH−/organic dianion was 2.0 : 1.0 : 8.0 : 1.5 in this experiment. The amino acid (L-CySH, L-Cys: 0.0375 mol) was dissolved in deionized water (50 ml). A solution containing Mg(NO3)2·6H2O (12.8 g, 0.05 mol) and Al(NO3)3·9H2O (9.38 g, 0.025 mol) in 100 ml deionized water was slowly added dropwise to the organic anion-containing solution with vigorous agitation under nitrogen. The value of the pH at the end of addition was adjusted to 10.5 for L-CySH LDHs and 10.0 for L-Cys LDHs by further addition of 2.4 mol L−1 NaOH solution. The resulting precipitate was aged at 65 °C under a nitrogen atmosphere for 24 h. The product was washed extensively with water, centrifuged and dried at room temperature under vacuum for a further 24 h. The product was subsequently isolated by filtration under suction and dried in a vacuum oven at room temperature under nitrogen atmosphere for 24 h. The amino acid (L-CySH, L-Cys: 0.0375 mol) was dissolved in deionized water (50 ml). The mixture was stirred under a nitrogen atmosphere for a further 24 h. Elemental analysis: Found (L-CySH LDHs) Mg 16.4%, Al 9.10%, S 7.04%, C 6.29%, N 2.44%, H 4.16%; Found (L-Cys LDHs) Mg 12.9%, Al 7.09%, S 7.04%, C 6.29%, N 2.23%, H 4.23%.

Oxidation of L-CySH LDHs by H2O2

0.5 ml H2O2 (3 wt.% solution in water) was added to a suspension of L-CySH LDHs (0.6 g, ca. 1 mmol) in deionized water (50 ml). The mixture was stirred under a nitrogen atmosphere for 1 h. The product was subsequently isolated by filtration under suction and dried in a vacuum oven at room temperature for 24 h. Elemental analysis: Found Mg 16.4%, Al 8.95%, S 4.21%, C 6.57%, N 1.93%, H 3.93%.

Oxidation of L-CySH LDHs by Br2

Br2 was added to a suspension of L-CySH LDHs (0.6 g, ca. 1 mmol) in deionized water (50 ml). The molar ratios of Br2/L-CySH were 0.5 and 3, respectively. The mixture was stirred under a nitrogen atmosphere for 1 h. The product was subsequently isolated by filtration under suction and dried in a vacuum oven at room temperature for 24 h. Elemental analysis: Found (the oxidation product with [Br2]/[CySH] = 0.5) Mg 17.5%, Al 9.98%, S 5.22%, C 6.35%, N 2.23%, H 4.16%; Found (the oxidation product with [Br2]/[CySH] = 3) Mg 16.5%, Al 9.72%, S 3.26%, C 3.42%, N 3.39%, H 4.23%.

Characterization

Powder X-ray diffraction (XRD) measurements were performed on a Rigaku XRD-6000 diffractometer, using CuKα radiation (λ = 0.154 nm) at 40 kV, 30 mA, a scanning rate of 5° min−1, a step size of 0.02° s−1, and a 2θ angle ranging from 3° to 70°. Fourier transform infrared (FT-IR) spectra were recorded using a Vector22 (Bruker) spectrophotometer in the range 4000-400 cm−1 with 2 cm−1 resolution. The standard KBr disk method (1 mg of sample in 100 mg of KBr) was used.

Analysis of metal element and sulfur was performed by ICP emission spectroscopy on a Shimadzu ICPS-7500 instrument. Carbon, hydrogen, and nitrogen analyses were carried out using an Elementalvario elemental analysis instrument.

13C solid-state magic-angle spinning nuclear magnetic resonance (MAS NMR) spectra were run on a Bruker AV300 spectrometer operating at a frequency of 75.467 MHz for 13C at a spinning rate of ~5000 Hz with a 5 s pulse delay.

Fourier-Transform Raman spectra were recorded, in backscattering geometry, using a Perkin-Elmer Spectrum 2000 NIR-FT Raman spectrometer (excitation wavelength 1064 nm, InGaAs detector). The laser power was set at 200–400 mW (at the sample) and typically 32 scans were accumulated. The spectral resolution was 4 cm−1.

Results and discussion

Structure of L-CySH and L-Cys intercalated LDHs

The powder XRD patterns of L-CySH LDHs and L-Cys LDHs are displayed in Fig. 1a and 1b, respectively, and the basal spacing and lattice parameters are listed in Table 1. In each case, the reflections can be indexed to a hexagonal lattice with R-3m rhombohedral symmetry, commonly used for the description of LDH structures. However, as is often the case, several of the (hkℓ) reflections disappear or broaden. The main diffraction peaks of L-CySH LDHs with LDH structure appear at 8.80° (003), 17.48° (006), 25.23° (009) and 61.15° (110) (Fig. 1a). The (003), (006), (009) and (110) peaks of L-Cys LDHs are observed at 6.37°, 12.06°, 19.22° and 61.34°, respectively (Fig. 1b). Well ordered (00) series indicates that the hydrotalcite-like structure of the host was maintained. The

Fig. 1 Powder XRD patterns of (a) L-CySH LDHs, (b) L-Cys LDHs, oxidation product of L-CySH LDHs by: (c) H2O2, (d) Br2 with [Br2]/[CySH] = 0.5 and (e) Br2 with [Br2]/[CySH] = 3.
XRD patterns for the L-CySH and L-Cys LDHs show expanded structure with broad (003) diffraction peaks at 1.00 and 1.39 nm, respectively (Table 1). The expanded interlayer separation is consistent with the intercalation of the organic anions within the gallery spaces of the LDHs. Assuming a thickness of 0.48 nm for the brucite layer, the gallery heights are approximately 0.52 and 0.91 nm for L-CySH and L-Cys LDHs, respectively.

Taking into account the solution pH and pKₐ, the distribution coefficient d can be calculated. The values of pKₐ of L-CySH are: pKₐ1 = 1.96 (–COOH), pKₐ2 = 8.18 (–NH₃⁺), pKₐ3 = 10.28 (R–SH). Based on the experimental condition for L-CySH LDHs with the aging pH at 10.5, the values of distribution coefficient d can be calculated as d₁ = 0.18%, d₂ = 37.53%, d₃ = 62.28%, thus L-CySH existed mainly as divalent anion \( \text{OOCCHNH}_2\text{CH}_2\text{SH}_2 \) during intercalation. The length of dianionic L-CySH is 0.56 nm, calculated by the method of molecular mechanics. Comparison of the length of dianionic L-CySH with a gallery height of 0.52 nm suggests that the dianionic L-CySH might exist obliquely to the LDH basal layer. A schematic model of the possible arrangement for L-CySH LDHs is shown in Fig. 2a.

In the case of L-Cys LDHs, the values of pKₐ are: pKₐ1 < 1.00 (–COOH), pKₐ2 = 2.10 (R–COOH), pKₐ3 = 8.02 (–NH₃⁺), pKₐ4 = 8.71 (R–NH₃⁺). The solution pH was 10.0 during aging and the values of calculated d are: d₁ = 6.43 × 10⁻¹⁰%, d₂ = 0.05%, d₃ = 4.88%, d₄ = 95.06%, as a result the L-Cys existed mainly as a divalent anion \( \text{OOCCHNH}_2\text{CH}_2\text{SSCH}_2\text{CHNH}_2\text{COO} \). The calculated length of L-Cys anion by the method of molecular mechanics is 0.97 nm, larger than the experimental value of gallery height of L-Cys LDHs (0.91 nm), indicating that the dianionic L-Cys might exist obliquely in the interlayer region as a monolayer with the two carboxyl groups of individual anions attaching electrostatically to hydroxide layers. On the basis of these data, a schematic representation of the L-Cys LDHs is shown in Fig. 2b.

The chemical compositions for the synthesized L-CySH and L-Cys LDHs are listed in Table 2. It should be noted that the water content was determined from the weight loss below 100 °C by thermogravimetric analysis; the interlayer organic content was calculated from the content by weight of sulfur by ICP; and the co-intercalated nitrate was obtained from the nitrogen content by elemental analysis. As shown in Table 2, the molar contents of organic guest for the synthesized L-CySH and L-Cys LDHs are equal to each other, possibly

### Table 1

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<tr>
<th>LDHs</th>
<th>d₀₀₋₀₀</th>
<th>d₀₀₋₆₀</th>
<th>d₀₀₋₉₀</th>
<th>d₁₁₀₋₀₀</th>
<th>Lattice parameter a/nm</th>
<th>Lattice parameter c/nm</th>
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<tr>
<td>L-CySH LDHs</td>
<td>1.004</td>
<td>0.507</td>
<td>0.353</td>
<td>0.152</td>
<td>0.304</td>
<td>3.012</td>
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<td>L-Cys LDHs</td>
<td>1.386</td>
<td>0.733</td>
<td>0.461</td>
<td>0.151</td>
<td>0.303</td>
<td>4.158</td>
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<tr>
<td>L-CySH LDHs + H₂O₂</td>
<td>1.202</td>
<td>0.595</td>
<td>—</td>
<td>0.152</td>
<td>0.304</td>
<td>3.605</td>
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<tr>
<td>L-CySH LDHs + 0.5Br₂</td>
<td>1.011</td>
<td>0.513</td>
<td>0.361</td>
<td>0.152</td>
<td>0.304</td>
<td>3.033</td>
</tr>
<tr>
<td>L-CySH LDHs + 3Br₂</td>
<td>1.067</td>
<td>—</td>
<td>—</td>
<td>0.152</td>
<td>0.304</td>
<td>3.201</td>
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### Table 2

<table>
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<th>Chemical Composition</th>
<th>Mg/Al Ratio</th>
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<td>Mg₁₆₋₇Al₁₆₋₇(OH)₂₋₆₋₀(C₂H₅NO₂S)₁₋₂₋₀(H₂O)₀₋₁₋₀</td>
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<tr>
<td>Mg₁₆₋₇Al₁₆₋₇(OH)₂₋₆₋₀(C₆H₁₀N₂O₄S₂)₁₋₂₋₀(H₂O)₀₋₁₋₀</td>
<td>2.0</td>
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<tr>
<td>Mg₁₆₋₇Al₁₆₋₇(OH)₂₋₆₋₀(C₂H₅NO₂S)₁₋₂₋₀(H₂O)₀₋₁₋₀</td>
<td>2.0</td>
</tr>
<tr>
<td>Mg₁₆₋₇Al₁₆₋₇(OH)₂₋₆₋₀(C₂H₅NO₂S)₁₋₂₋₀(H₂O)₀₋₁₋₀</td>
<td>2.0</td>
</tr>
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due to their divalent anions. The experimentally determined Mg/Al molar ratio of L-CySH and L-Cys LDHs is approximately equal to that of the Mg/Al input, indicating that the coprecipitation process was rather complete.

The FT-IR spectra of the pristine L-CySH and L-Cys, as well as the intercalated compounds L-CySH LDHs and L-Cys LDHs are presented in Fig. 3. The absorption bands at 3180 and 2972 cm$^{-1}$ in Fig. 3a, the strong band at 3028 cm$^{-1}$ in Fig. 3b along with several weak bands in the range 2950–2200 cm$^{-1}$ are attributed to the alkyl C–H amine N–H stretching vibrational absorptions. Furthermore, the strong asymmetric stretching vibration peak of R–COO$^-$ at 1586 cm$^{-1}$ (Fig. 3a) and 1585 cm$^{-1}$ (Fig. 3b) as well as symmetric stretching at 1394 cm$^{-1}$ (Fig. 3a) and 1408 cm$^{-1}$ (Fig. 3b) were observed. In particular, the S–H stretching vibration at 2552 cm$^{-1}$ (Fig. 3a) was absent in the spectra of L-CySH LDHs (Fig. 3c), indicating that the interlayer L-CySH existed as divalent anion. The R–COO$^-$ asymmetric and symmetric stretching vibration absorptions were observed at 1577 and 1406 cm$^{-1}$ (Fig. 3c), as well as at 1561 and 1398 cm$^{-1}$ (Fig. 3d), respectively. The co-intercalated nitrate anion in L-CySH LDHs gives an intense absorption band at 1385 cm$^{-1}$ (Fig. 3c). However, this band was not observed in the spectra of L-Cys LDHs (Fig. 3d), which might be overlapped by the symmetric stretching vibration of R–COO$^-$. The lattice vibration of metal cations Al$^{3+}$ and Mg$^{2+}$ are noted at 667 cm$^{-1}$ (Fig. 3c), and at 659 cm$^{-1}$ (Fig. 3d). In addition, the absorption bands both at 447 cm$^{-1}$ are assigned to the M–O–M and O–M–O skeletal stretching and bending vibrations.

**Oxidation of L-CySH LDHs by H$_2$O$_2$**

The oxidation of L-CySH LDHs was carried out by adding H$_2$O$_2$ to the suspension of L-CySH LDHs in water. Fig. 4 displays the FT-Raman spectra of the as-synthesized L-CySH LDHs and L-Cys LDHs, and the oxidation product of L-CySH LDHs. After the oxidation of L-CySH LDHs by H$_2$O$_2$, a new intense absorption band at 504 cm$^{-1}$ was observed (Fig. 4c) compared with the as-synthesized L-CySH LDHs (Fig. 4a). This is attributed to the characteristic vibrational absorption of the disulfide linkage [S–S], which was also observed in the Raman spectrum of L-Cys LDHs (505 cm$^{-1}$, Fig. 4b), indicating that the interlayer L-CySH has transformed to L-Cys by the reaction with H$_2$O$_2$ diffusing into the LDHs interlayer. This will be further confirmed by the XRD and NMR data in the next section. Analogous to the infrared spectra, the broad absorption band around 3300–3200 cm$^{-1}$ in the Raman spectra is assigned to stretching vibration of hydroxyl groups and water molecules. Characteristic alkyl C–H stretches were observed in the region 2950–2900 cm$^{-1}$ and the R–COO$^-$ asymmetric and symmetric stretches at 1566 and 1428 cm$^{-1}$ (Fig. 4a), 1601 and 1402 cm$^{-1}$ (Fig. 4b), 1588 and 1397 cm$^{-1}$ (Fig. 4c) for the three materials, respectively. Several absorptions in the range 1300–800 cm$^{-1}$ are assigned to the C–C stretching vibration. Peaks at 681 and 552 cm$^{-1}$ (Fig. 4a), 663 and 555 cm$^{-1}$ (Fig. 4b), 660 and 552 cm$^{-1}$ (Fig. 4c) are attributed to the metal–oxygen modes of the LDH sheets, respectively.

Oxidation treatment of the L-CySH LDHs by H$_2$O$_2$ results in an increment in the interlayer spacing. The powder XRD pattern for the oxidation product of L-CySH LDHs shows the characteristic reflections corresponding to an interlayer spacing of 1.20 nm (Fig. 1c, Table 1). This basal spacing value is smaller than that of the as-synthesized L-Cys LDHs (1.39 nm). This is possibly related to the different arrangement of L-Cys in the interlayer region. Additionally, the well ordered (00l) series indicates that the lamellar structure of LDH sheets is maintained during the oxidation reaction. The peaks around 11.8° and 22.9° are assigned to the (003) and (006) reflections of CO$_3^{2-}$-LDH, implying that CO$_3^{2-}$ co-entered into the interlayer during the oxidation process. This will be discussed further below.

The gallery height of the oxidation product of L-CySH LDHs is 0.72 nm, which is smaller than the calculated length of L-Cys (0.97 nm). The results suggest that the oxidation product L-Cys might be accommodated obliquely to the host...
layer, in which the oblique angle is ~48° between the host layer and the L-Cys molecular chain. The possible arrangement is shown in Fig. 2c.

Elemental analysis of the oxidation product of L-CySH LDHs by H₂O₂ gives a chemical composition, Mg₀.₆₇Al₀.₃₃(OH)₂(C₆H₁₀N₂O₄S₂)₀.₀₇(CO₃)₀.₁₋₀.₆H₂O (Table 2). The organic content of the oxidation product is one half of the as-synthesized L-Cys LDHs. However, no nitrate anions, but carbonate anions were found interlayer. After the oxidation reaction, two molecules (C₆H₁₀NO₄S₂)₂ transformed to one (C₆H₁₀N₂O₄S₂)₂, resulting in the one half decrease of interlayer negative charge. Thus, other inorganic anions (generally CO₃²⁻ for its high affinity to LDHs sheets) must be introduced into the interlayer region in order to compensate for the positive charge of the LDH sheets. The results obtained by elemental analysis are in accordance with those of XRD. The interlayer transformation of L-CySH to L-Cys by H₂O₂ is shown in Scheme 1.

Supporting evidence for oxidation of the intercalated L-CySH by H₂O₂ was provided by solid-state ¹³C NMR spectroscopy. The spectrum of the L-Cys LDHs displays resonances at 180.7 ppm (COO⁻), 56.9 ppm (CHN) and 32.1 ppm (CH₂) (Fig. 5a). An additional peak at 170.5 ppm is due to some adsorbed carbonate. After the oxidation by H₂O₂, the chemical shift of the resonance absorption lines moved upfield and were observed at 179.3 ppm (COO⁻), 55.8 ppm (CHN) and 29.4 ppm (CH₂) (Fig. 5b), respectively, which are consistent with the presence of the as-synthesized L-Cys LDHs [180.0 ppm (COO⁻), 56.2 ppm (CHN) and 29.3 ppm (CH₂)] (Fig. 5c). The three spectra also contain several weak resonances at 40, 70 and 164 ppm, indicating that some unknown species formed during the intercalation reaction, which may be caused by partial thermolysis of L-CySH and L-Cys under synthesize condition due to their facile decomposition.

The comparative experiment in bulk reaction, i.e., the oxidation of L-CySH by H₂O₂ was also carried out in water solution, and Fig. 4d displays the FT-Raman spectrum of the oxidation product. An intense absorption band of the disulfide linkage [S–S] at 498 cm⁻¹ (Fig. 4d) was observed, indicating that cystine was the oxidation product. Darkwa et al.²⁴ and Gilbert et al.³³ have studied the oxidation of L-CySH by H₂O₂ in solution in detail, and reported that this reaction occurs via free radical mechanism. The predominant pathway for free-radical mediated oxidation of thiols is via the dimer: cystine, which is extremely insoluble and is rapidly precipitated. The results in this work are in accordance with those previously reported.

Oxidation of L-Cys LDHs by Br₂

The FT-Raman spectra of the oxidation products of L-Cys LDHs by Br₂ with [Br₂]/[CySH] = 0.5 and 3, are shown in Fig. 6a and 6b, respectively. In both the cases, the characteristic vibrational absorption of the disulfide linkage [S–S] at 504 cm⁻¹ was not observed, indicating that no cystine was produced under the two conditions. However, an increase in intensity of the bands centered at 1042 cm⁻¹ (Fig. 6a) and 1041 cm⁻¹ (Fig. 6b) was observed, which is related to the formation of the sulfonic group in the oxidation products. The bands at 552 cm⁻¹ (Fig. 6a and Fig. 6b) are due to the M–O–H

Scheme 1 Schematic representation of the oxidation process for the intercalated l-CySH by H₂O₂.

Oxidation of L-CySH LDHs by Br₂

The FT-Raman spectra of the oxidation products of L-CySH LDHs by Br₂ with [Br₂]/[CySH] = 0.5 and 3, are shown in Fig. 6a and 6b, respectively. In both the cases, the characteristic vibrational absorption of the disulfide linkage [S–S] at 504 cm⁻¹ was not observed, indicating that no cystine was produced under the two conditions. However, an increase in intensity of the bands centered at 1042 cm⁻¹ (Fig. 6a) and 1041 cm⁻¹ (Fig. 6b) was observed, which is related to the formation of the sulfonic group in the oxidation products. The bands at 552 cm⁻¹ (Fig. 6a and Fig. 6b) are due to the M–O–H
bending deformation vibration. In comparison with the oxidation reaction carried out in the confined space of the LDHs, the oxidation of L-CySH in solution using identical experimental conditions but in the absence of the LDHs has also been investigated. Fig. 6c displays the Raman spectrum of oxidation product of L-CySH in solution by Br$_2$ with [Br$_2$]/[CySH] = 0.5. An intense absorption band at 496 cm$^{-1}$ (Fig. 6c) is attributed to the disulfide linkage [S–S] vibrational absorption of cystine. In contrast, the Raman spectrum of the oxidation product of L-CySH in solution by Br$_2$ with [Br$_2$]/[CySH] = 3 shows no characteristic vibrational absorption of the disulfide linkage, but a band at 1046 cm$^{-1}$ (Fig. 6d) which is assigned to the sulfonic group is observed. The results above indicate that the oxidation reaction of L-CySH in the confined region of LDHs is quite different from that in bulk reaction. In aqueous solution, the amount of Br$_2$ has a remarkable influence on the oxidation product of L-CySH, viz., cystine is obtained with a lower Br$_2$ content while higher Br$_2$ gives cysteic acid. However, in the case of the reaction occurring in the confined space of LDHs, only cysteic acid is produced, independent of the amount of oxidant. This will be further discussed in the next section.

Fig. 7 displays the FT-IR spectra of the two oxidation products of L-CySH by Br$_2$: (a) interlayer product with [Br$_2$]/[CySH] = 0.5, (b) interlayer product with [Br$_2$]/[CySH] = 3, (c) oxidation product in solution with [Br$_2$]/[CySH] = 0.5, (d) oxidation product in solution with [Br$_2$]/[CySH] = 3.

As a result, only cysteic acid is obtained regardless of the quantity of oxidant Br$_2$. This is might be related to reason that the diffusion rate of reaction compared with the rate of diffusion of Br$_2$. Darkwa et al. investigated the reaction between cysteine and bromine in bulk reaction system in detail, and found that the possible products include cystine, cysteinesulfinic acid and cysteic acid according to the variation of molar ratio of bromine/cysteine:24

\[
\text{Br}_2 + \text{Cys-SH} + \text{H}_2\text{O} \rightarrow \text{Cys-SOH} + 2\text{Br}^- + 2\text{H}^+ \quad (1)
\]

\[
\text{Cys-SOH} + \text{Cys-SH} \rightarrow \text{Cys-S-S-Cys} + \text{H}_2\text{O} \quad (2)
\]

\[
\text{Cys-S-S-Cys} + 2\text{H}_2\text{O} \rightarrow 2\text{Cys-SOH} + 2\text{H}^+ + 2e^- \quad (3)
\]

\[
\text{Br}_2 + \text{Cys-SOH} + \text{H}_2\text{O} \rightarrow \text{Cys-SO}_2\text{H} + 2\text{Br}^- + 2\text{H}^+ \quad (4)
\]

\[
\text{Br}_2 + \text{Cys-SO}_2\text{H} + \text{H}_2\text{O} \rightarrow \text{Cys-SO}_3\text{H} + 2\text{Br}^- + 2\text{H}^+ \quad (5)
\]

However, the reaction is rather different in the restricted region between LDHs layers. The oxidation product of cysteine is only cysteic acid, independent of the quantity of Br$_2$. This is might be related to reason that the diffusion process of Br$_2$ between the LDH layers is the rate-determining step for the interlayer oxidation reaction of L-CySH. At the first stage, Br$_2$ molecules diffuse into the interlayer edges of LDHs and oxidize cysteine to cystine. Then, further oxidation of cystine to cysteic acid occurs owing to its rather faster rate of reaction compared with the rate of diffusion of Br$_2$. As a result, only cysteic acid is obtained regardless of the quantity of oxidant Br$_2$. The reaction process is presented in Scheme 2.
The solid-state $^{13}$C NMR spectra of the oxidation products of L-CySH LDHs by Br$_2$ are shown in Fig. 8. Oxidation treatment with [Br$_2$]/[CySH] = 0.5 or [Br$_2$]/[CySH] = 3 results in an increase in intensity of the resonance at 52.4 ppm (Fig. 8a) and 52.5 ppm (Fig. 8b), which is attributed to the superposition of δ (CHN) and δ (CH$_2$SO$_3^-$), demonstrating the formation of cysteic acid in both two cases. However, a weak resonance at 32.3 (CH$_2$S$^-$) was also observed in Fig. 8a, indicating that a small quantity of L-CySH in LDHs remained unreacted because of the low Br$_2$ content.

The powder XRD patterns of the oxidation products of L-CySH LDHs by Br$_2$ show the characteristic reflections corresponding to an interlayer spacing of 1.01 nm (Fig. 1d, Table 1, [Br$_2$]/[CySH] = 0.5) and 1.07 nm (Fig. 1e, Table 1, [Br$_2$]/[CySH] = 3), respectively. The main diffraction peak ($d_{003} = 0.79$ nm) of CO$_3$-LDH was also observed in Fig. 1d. The result is consistent with the chemical composition given by elemental analysis, Mg$_{0.65}$Al$_{0.33}$(OH)$_2.00$(C$_3$H$_5$NO$_2$S)$_{0.12}$-(C$_3$H$_5$NO$_5$S)$_{0.02}$(CO$_3$)$_{0.02}$?0.5H$_2$O (Table 2). It can be seen that the XRD pattern of the oxidation product with [Br$_2$]/[CySH] = 3 was not well defined (Fig. 1e). A peak at 11.2° 2θ was observed, which could be attributed to the (003) reflection of CO$_3$-LDH, while the strong peak at 22.3° 2θ might be assigned to the superposition of (009) reflection of the oxidation product and (006) reflection of CO$_3$-LDH. However, this does not accord with the result of elemental analysis that no other inorganic anions were found in this material (Table 2). The not well-defined XRD pattern indicates that the interlayer reaction with [Br$_2$]/[CySH] = 3 was rather complicated. In addition, a decrease in stacking sequence and crystallinity occurred, resulting from partial destruction of the LDHs matrix under acidic solution conditions (due to the formation of proton, as shown in eqn. (1)–(5). In order to confirm this point, the contents of Mg and Al in solution after the interlayer oxidation reaction with bromine were determined. It was found that the concentrations of both Mg and Al were rather low and can be neglected with [Br$_2$]/[CySH] = 0.5; while in the case of [Br$_2$]/[CySH] = 3.0, about 8.9% of the total host hydroxide dissolved during the reaction. Moreover, the molar ratio of Mg/Al was close to 4, higher than that of the resulting solid sample, indicating that more Mg$^{2+}$ in the host layer was dissolved than Al$^{3+}$, due to the larger solubility product constant ($K_{sp}$) of Mg(OH)$_2$ than that of Al(OH)$_3$. This demonstrates that the LDHs matrix is not suitable for the occurrence of interlayer reactions under acidic conditions. Nevertheless, the diffraction maximum between 60 and 62° 2θ (Fig. 1e) still can be observed, indicating the conservation of the lamellar structure with no substantial structural collapse during the interlayer oxidation reaction. As a result, this layered material can be employed as the basis of a novel “molecular reactor” to impose restricted chemical reaction leading to enhanced control of product distribution.

**Fig. 8** $^{13}$C MAS NMR spectra of the oxidation products of L-CySH LDHs by Br$_2$: (a) [Br$_2$]/[CySH] = 0.5, (b) [Br$_2$]/[CySH] = 3. Asterisks indicate spinning side-bands.
Conclusions

In this paper, two amino acid intercalated LDHs materials, containing L-CySH and L-Cys, have been prepared by direct synthesis and characterized by XRD, FT-IR and elemental analysis. Analysis by FT-IR shows that both the interlayer guests are divalent anions.

FT-Raman, FT-IR, and $^{13}$C MAS NMR measurements allow a detailed understanding of the oxidation process for L-CySH between sheets of LDHs. In the case of H$_2$O$_2$ as oxidant, Raman and NMR data demonstrate the interlayer oxidation transformation of L-CySH to cystine, just the same as the reaction in aqueous solution. However, the XRD results show that the basal spacing of the oxidation product is smaller than that of the as-synthesized L-Cys LDHs, which might be due to their different interlayer arrangement resulting from different organic anion density in the interlayer. However, the oxidation of L-CySH LDHs by Br$_2$ produced the highest oxidation product: cysteic acid, regardless of the difference in bromine/cysteine ratio ($[\text{Br}_2]/[\text{Cys}] = 0.5$), due to the low rate of diffusion of Br$_2$ between the LDH sheets. This is much different from the bulk reaction between L-CySH and Br$_2$, whose product is extraordinarily dependent on the bromine/cysteine ratio.

Therefore, this work provides the way for prospective application of LDHs as novel reactors for confined chemical reactions, and new opportunities for further research into molecular reactions on the nanoscale of inorganic hybrids and for their applications in catalysis and separation science.

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