ADSORPTION OF SOME AMINO ACIDS BY CHRYSOTILE.

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Abstract

Adsorption of 10 types of amino acids (Gly, Ala, Val, Leu, Ile, Pro, Ser, Asp, Glu, and Phe) has been studied using chrysotile, which is a mineral belonging to the serpentine group. The adsorption of Asp and Glu by chrysotile was strong while Gly, Ala, Val, Leu, Ile, Pro, Ser and Phe were hardly adsorbed by chrysotile. It was found that adsorption behavior of these amino acids was mainly affected by the dissociation behavior of the adsorbent and the adsorbates, and also by the solubility of the adsorbate into water. Around at pH 8, chrysotile would have positive charges, and Asp and Glu possessed negative charges. Therefore, Asp and Glu could be adsorbed well on chrysotile by electrostatic interaction. It was shown that the adsorption of amino acids was inversely correlated with their solubility into water. The adsorption behavior of amino acids by chrysotile could be explained on the basis of the dissociation of amino acids and their solubility into water. This trend of the adsorption behavior of amino acids by chrysotile was similar to that by kaolinite [9]. This fact suggested that chrysotile would possess the similarity of crystal structure to kaolinite. Additionally, chiral separation of D- and L-amino acid would be difficult by chrysotile.

(Keywords) adsorption, amino acid, chrysotile, discrimination between optical isomers.

1. Introduction

Clay minerals have several functions such as catalytic and adsorptive activities for organic compounds [1, 2]. The relationships between the adsorptive ability and catalytic activity of clay minerals for bio-molecules have been investigated [3 – 8]. Hedges and Hare (1987) investigated adsorption of 15 amino acids by montmorillonite and kaolinite in diluted aqueous solutions [9]. The adsorption studies showed that basic amino acids were adsorbed strongly by both montmorillonite and kaolinite and acidic amino acids were only adsorbed well by kaolinite. Neutral amino acids were hardly adsorbed by either montmorillonite or kaolinite. Siffert and Naidja (1992) investigated selective adsorption of racemic Asp and Glu using a natural montmorillonite, where Mg(II) cation was enriched in the silicate layer [10]. An isotherm showed that the adsorption of D-Asp was stronger than that of L-Asp. On the other hand, the adsorption of L-Glu was stronger than that of D-Glu. Montmorillonite might have an asymmetric structure by exchanging an Al with Mg in octahedral site of the layer [11]. Therefore, Mg enriched montmorillonite could result in the selective adsorption of optical isomers. For instance, Hashizume et al. (2000) showed the discrimination between D-alanyl-D-alanine and L-alanyl-L-alanine by allophane [12]. Allophane has a hallow spherule structure and its outer diameter is from 3.5 to 5.5 nm and several perforations on the surface. Some Al-OH or Si-OH groups that would be related to the adsorption site for amino acids are exposed on the wall of the perforation. Allophane generally causes an aggregate. The discrimination between di-alanine enatiomers was expected on the basis of the shape of the perforation, the OH groups, and the formation of the aggregate. It is known that kaolinite has levorotatory and dextrorotatory crystals as well as α-quartz [13]. It was shown by theoretical calculation that one of these types of kaolinite existed slightly more frequently than the other types [14]. If amino acids were synthesized from the precursors in the presence of such a type of kaolinite, the chiral discrimination of D- or L-amino acid would have been occurred. Moreover, it was shown that kaolinite could adsorb D- or L-amino acid selectively [14, 15]. Further, an edge of kaolinite might be capable to discriminate D- and L-amino acids, where Al-OH and Si-OH groups existed at the edge of kaolinite. Organic molecules could be adsorbed on the OH group of the edge by hydrogen bonding. It had been shown that the edge might have a chiral center by a specific arrangement of the OH group [16].

Concerning the origin of life, the surface of the early earth had been mainly covered with basaltic rocks, of which mainly involved Mg, Si and Al [17]. According to the chemical composition analyses of the early crust, not only clay minerals such as kaolinite, pyrophyllite, and montmorillonite consisting of the system of Al_2O_3-SiO_2-H_2O, but also serpentine and talc consisting of the system of MgO-SiO_2-H_2O could have been formed from the basaltic glasses or rocks on the surface of the early earth. It would be necessary and important to investigate the relationship between the characteristics of clay minerals involving of MgO, SiO_2 and OH groups and the adsorption of bioorganic compounds by the minerals. Clay minerals consisting of MgO, SiO_2 and H_2O mainly belong to serpentine group (chrysotile, lizardite and anitgorite) and talc. They are generally formed by weathering of olivine, pyroxine, and basaltic glass.

Serpentine group is a layered silicate, and involves three different types, that is to say chrysotile,

lizardite, and antigorite. Serpentine group minerals are composed of a tetrahedral (Si site) and an octahedral (Mg site) sheet. The chemical composition of serpentine group is ideally $Mg_3Si_2O_5(OH)_4$. Chrysotile consists of tubular fibers with such chemical composition, where the layer sheet rolls up; the octahedral sheet of Mg site is larger than the tetrahedral sheet of Si site [18]. Lizardite forms a plane sheet. In the case of natural lizardite, cations such as Fe^{3+} or Al^{3+} are included in the octahedral sheet. Thus, the sheet of lizardite is not curved. Antigorite is also a plane sheet silicate [18]. The octahedral sheet of antigorite is continuous as shown in Fig. 1. In the period (A) in Fig. 1, the octahedral sheet is not bonded to the just above octahedral sheet but the just below tetrahedral sheet, while in the period (B) the octahedral sheet of the bottom is not bonded to the just below tetrahedral sheet but the the just above tetrahedral sheet. In other words, an octahedral sheet is bonded to one adjacent tetrahedral sheet of one side in one period, and in another period the octahedral sheet is bonded to the another adjacent tetrahedral sheet (Fig. 1). Therefore, antigorite forms a plane sheet. The crystal structure of serpentine group minerals is similar to that of kaolinite. Kaolinite is a layered silicate and is composed of the stratification of the octahedral (Al site) and tetrahedral (Si site) sheet. In order to balance the charges of the tetrahedral sheet with those of the octahedral sheet, the octahedral sheet is not filled by Al^{3+}, that is to say that there are vacant sites in the octahedral sheet. For that reason, kaolinite does not show a tubular fiber structure, Kaolinite has dextrorotatory and levorotatory forms in its structure, because of the distribution of the vacant sites in the octahedral sheet [14]. Also, it was expected that the edge of kaolinite might have chiral centers by presence of hydroxyl groups [16]. In the serpentine group, chrysotile and lizardite are almost of the same crystal structure as kaolinite. Chrysotile has almost the ideal chemical composition $Mg_3Si_2O_5(OH)_4$, and forms the tubular fiber. Therefore, chrysotile was chosen in the present research.

Adsorption of amino acids by chrysotile has been investigated in this study. Chrysotile was synthesized. Fine particles of synthesized chrysotile, which were fractionated smaller than 2 μm, were used for the adsorption experiments. Ten amino acids, glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), serine (Ser), phenylalanine (Phe), aspartic acid (Asp) and glutamic acid (Glu), were chosen. These amino acids could have been formed under the prebiotic conditions [19]. In addition, these have already been found in meteorites [20]. Wong (1988) proposed the hypothesis for the co-evolution of the genetic code [21], in which 6 to 10 amino acids might have been used for 61 codones in the first stage of origin of the genetic code. Based on these facts, 10 amino acids were examined in this work. Furthermore, it was investigated whether chrysotile could discriminate D- and L-amino acids by adsorption or not.

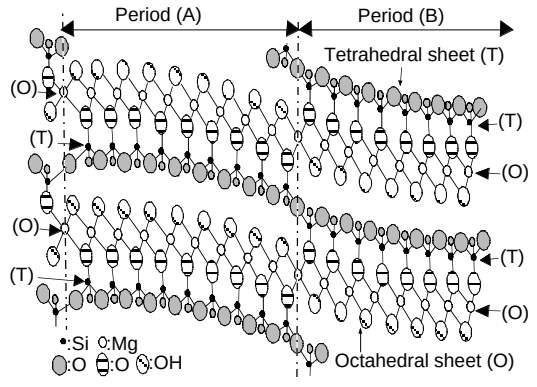

Fig. 1 Schematic illustration of antigorite.

2. Experimental

Chyrsotile used was prepared by hydrothermal synthesis, in which MgO and SiO_2 (reagent grade, Wako Pure Chemical Industries Ltd) were mixed according to the chemical composition of chrysotile (i.e. $MgO:SiO_2$ = 3:2 in molar ratio). Five g of the mixture of MgO and SiO_2, and 5 g of deionized water were put into a reaction vessel. The reaction vessel was placed in an oven and was heated at 200 °C for 6 weeks. The product was analyzed by an XRD to confirm that chrysotile was synthesized. Chrysotile was ground softly, and then it was dispersed in deionized water. Chrysotile particles smaller than 2 μm were fractionated by the difference of a sedimentation ratio. After the fractionation, chrysotile was treated using 10 % of H_2O_2 at 100 ℃, and dialyzed with deionized water. Chrysotile fractionated as adsorbent was dried at 60 °C in an oven. The dried chrysotile was confirmed by the XRD which did not include any other materials.

Nine racemic amino acids (Ala, Val, Leu, Ile, Pro, Ser, Phe, Asp, and Glu) and Gly were used of the reagent grade (Wako Pure Chemical Industries Ltd). Amino acid solutions containing 5 mmol/l amino acid were prepared. It was added 7 ml of amino acid solution and 150 mg chrysotile in a glass bottle with a stopper. After the shaking of the bottle for 65 h, pH of the suspension was measured. The pH was 8.0 ± 1.0. The suspension was centrifuged to separate supernatant and chrysotile. The extents of amino acid in the initial solution and the supernatant after the reaction were measured 5 – 7 times by a total organic carbon analyzer (Shimadzu TOC-5000A). Concentrations of amino acids in the initial solution and the supernatant were obtained by their average carbon contents. Adsorption capacity of amino acids by chrysotile was estimated by the concentration of amino acids in the initial solution and the supernatant. For the confirmation of the adsorption capacity by chrysotile, the adsorption treatment was performed 3 - 4 times to use the fresh chrysotile and amino acid solutions.

For discrimination between D- and L-amino acids by chrysotile, the extent of amino acids in supernatant after adsorption by chrysotile was

measured by a high performance liquid chromatogram (Hitachi 4200 series) on a Shimpack FC-ODS using an eluent containing 0.1% trifruoracetic acid at 0.9 ml/min with a circular dichroism detector Jusco CD-2015 (HPLC-CD). The CD detector is capable to monitor ultraviolet (UV) absorption as well as CD at 220 nm. The initial concentrations of amino acid before adsorption were also measured by HPLC-CD to confirm that the same concentrations of D- and L-amino acid existed. It was confirmed that there were no peaks found by CD detector for the initial solutions.

3. Results and discussion.

3.1 Adsorption of 10 amino acids

The average adsorption capacities of chrysotile for 10 amino acids were depicted in Fig. 2, where the error bars were indicated. Adsorption of Asp was highest and adsorption of Glu was second highest among 10 amino acids. Except for Asp and Glu, there is a trend that amino acids possessing large molecular weights are adsorbed on chrysotile strongly . For instance, Gly and Ala were less adsorbed on chrysotile, but Phe was adsorbed strongly. Ile and Ser may be exceptional (see Table 1).

Adsorption is generally related to the abundance of charges of an adsorbent and an adsorbate as well as the solubility of the adsorbate into water. Dissociation constants of amino acids used were shown in Table 1. The values of pH of the suspension after adsorption treatment were 7 to 9. Thus, the amino acids exist as neutral or slightly negatively charged forms. Asp and Glu would possess two negative charges. On the other hand, the dissociation behavior of the surface of chrysotile is not known. The dissociation constant for silanol group (pKa) at the silicate surface is 9.86 [22]. At pH 8, silanol group located on the edge of chrysotile may slightly have a positive charge such as $Si\text{-}OH_2^+$. Therefore, since Asp and Glu have the negative charges these will be capable to be more strongly adsorbed on chrysotile than other amino acids.

There is a trend that molecules which are readily adsorbed possess low solubility into water within a water-adsorbent system. Generally, a molecule possessing high solubility into water does not be adsorbed strongly by an adsorbent, while the molecules with low solubility into water have a high adsorption capacity by the adsorbent. The solubility of 10 amino acids into water was shown in Table 1. The relationship between solubility into water and amino aids and the relationship between adsorption and amino acids was also added in Fig. 3. It was found that the strength of adsorption of amino acids are inversely correlated with the solubility of amino acid into water (Fig. 3), while Gly and Glu were exceptions. In the case of Glu, the effect on the negative charges of Glu may be stronger than that on solubility into water. The determination of the

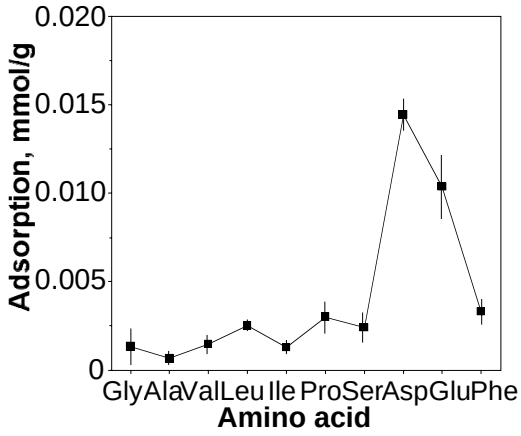

Fig. 2 Adsorption of individual amino acids by chrysotile.

Table 1 Dissociation constant [23], solubility to water [25] and molecular weight [24] of 10 amino acids.

Amino acid	Dissociation constant			Solubility to water	Molecular
	pK_1(α-COOH)	pK_2(α-NH_3^+)	pK_R(β-COOH)	g/100g(water)	weight (g)
Gly	2.35	9.78		18.4	75.07
Ala	2.35	9.87		13.55	89.09
Val	2.29	9.74		6.38	117.2
Leu	2.33	9.74		0.93	131.2
Ile	2.32	9.76		2.079	131.2
Pro	2.95	10.65		1.55	115.1
Ser	2.19	9.21		4.118	105.1
Asp	1.99	9.90	3.90	0.629	132.1
Glu	2.10	9.47	4.07	1.693	147.1
Phe	2.16	9.18		1.29	165.2

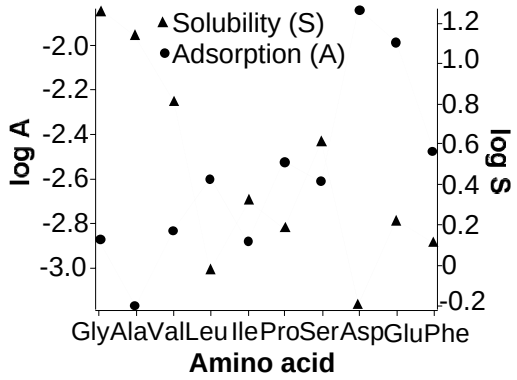

Fig. 3 Relationship between solubility to water [23] and adsorption of individual amino acids. Solid circles show adsorption (A) and triangles show solubility (S).

capacity of adsorption of Gly involved a fairly large error as shown in Fig. 2. The relationship between the adsorption and the solubility into water for Gly may be correlated with the fact that the relatively large error may be involved for the determination of adsorption. Generally, the solubility of large amino acids into water is low and that of small amino acids is high. Therefore, it may be true that large amino acids in size could be more readily adsorbed than small amino acids. The adsorption of an amino acid by chrysotile would be affected well on the dissociation behavior of the amino acid and the solubility to water.

Chrysotile possesses the same type crystal structure as kaolinite. Chrysotile has Mg cation in the octahedral site, while kaolinite has Al cation in the octahedral site. Adsorption of 15 amino acids by kaolinite was reported [9]. According to the comparison between the adsorption of 9 amino acids (i.e. Gly, Ala, Val, Leu, Ile, Ser, Asp, Glu and Phe) by chrysotile with that by kaolinite, both of chrysotile and kaolinite adsorbed Asp and Glu strongly, while their clay minerals hardly adsorbed Ala, Val, Ile and Leu etc. The trend for adsorption by chrysotile was similar to that by kaolinite, where the initial concentration of amino acids used in the present study was much higher than that used in the previous study [9]. That trend of adsorption by chrysotile and kaolinite will depend on the similarity of the crystal structure of chrysotile to that of kaolinite.

3.2 Possibility of discrimination between D- and L-amino acids

Chromatograms using the CD and UV detectors for supernatants including amino acids after the adsorption experiments were shown in Fig. 4. HPLC peaks in all the chromatograms appeared using the UV detector, while any peaks were not detected clearly using the CD detector. The fact that the adsorption of amino acids by chrysotile was not strong might be the reason that it was difficult to detect any peaks on the chromatogram using CD. Nevertheless, the chromatograms for CD of Pro and

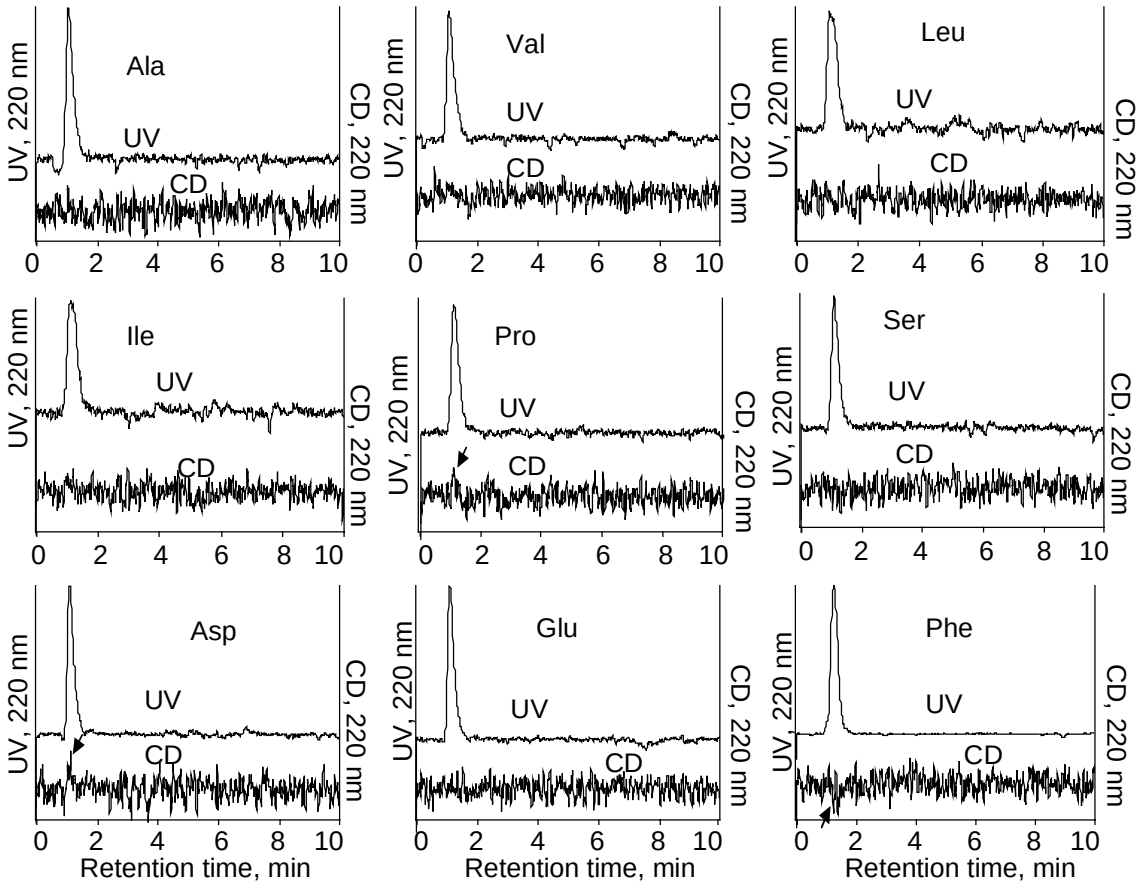

Fig. 4 Chromatograms for UV and CD of individual amino acids except gly.

Asp might indicate a small positive peak and the chromatogram for CD of Phe might show a negative peak as shown by the arrow in Fig. 4, though these data are ambiguous hidden in signal noises. This may be due to that L-Pro, L-Asp, and D-Phe might be concentrated in supernatant so that D-Pro, D-Asp, and L-Phe might be adsorbed by chrysotile more strongly than L-Pro, L-Asp and D-Phe.

If the discrimination between D- and L-amino acids by chrysotile occurs, chrysotile should have specific adsorption sites for the discrimination. It is considered that such the specific adsorption sites can be formed in chrysotile structure because of the following reasons. Chrysotile hardly has any surface charges, since Mg cation is located in the octahedral site without any vacant sites [2]. Chrysotile, however, has positive or negative charges on the edge according to the dissociation of OH group [2]. It is expected that the amount of an amino acid is adsorbed on an edge of chrysotile. The size of the octahedral sheet for Mg cation is slightly larger than that of the tetrahedral sheet for Si cation in the chrysotile structure. Because of this reason, the chrysotile sheet forms spiral cylindrical structure as the outer surface of chrysotile is of the octahedral sheet. The inner diameter of the cylinder of chryositle is 5 to 10 nm [18]. A scheme for the adsorption site for discrimination between D- and L-amino acid is shown in Fig. 5. When some Si-OH groups of the edge are dissolved into water in Fig. 5 (a), the edge of the tetrahedral sheet will have positive charges. If the edge in the cylinder is formed as shown in Fig. 5 (b), an L-amino acid would be likely adsorbed on the edge of the cylinder. The side chain of the amino acid will be placed on or near SiO_2 surface of the adjacent layer, because there are any charges on the chrysotile surface except the edge. The carboxyl and amino group of the amino acid will be bonded to $Si\text{-}OH_2^+$ of the tetrahedral sheet and $Mg\text{-}O^-$ in the octahedral sheet by the hydrogen bonding, respectively. Therefore, the adsorptive formation of the amino acid to chrysotile as shown in Fig 5 (b) would be expected. However, chrysotile involves both the clockwise and anticlockwise spiral structures in the almost the same ratio. Furthermore, the existence of the alternative structure of which the edge prefers to adsorb D-amino acid can be naturally supposed. Therefore, it is not straightforwardly determined whether chrysotile prefers to adsorb D-Pro, D-Asp, and L-Phe or their enantiomers. On the CD chromatogram, a small peak, which was showed by the arrow, appeared in the same time as the peak on UV chromatogram (Fig. 4). However, the peaks using the CD detector was so small that it may not be distinguished from noise. Thus, it is difficult to conclude whether chrysotile possesses capability for the discrimination of one of the enantiomers of an amino acid from the other one. Further investigations will be necessary.

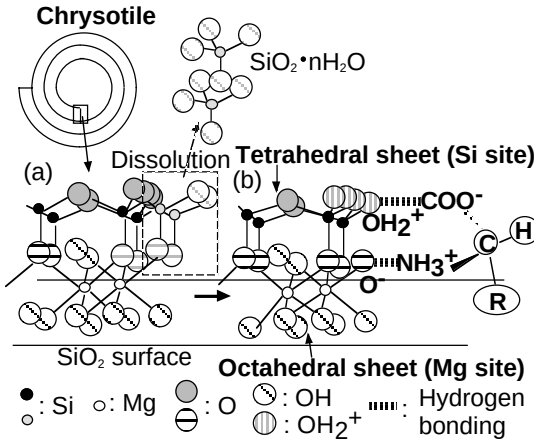

Fig. 5 A possible schematic illustration of a selective adsorption site on the edge of chrysotile is shown in (a). SiO_4 tetrahedron is dissolved into water from the edge more easily than MgO. After the dissolution of SiO_4 tetrahedron, the structure of the edge would become a hydrated form as shown in (b), where $Si\text{-}OH_2^+$ and $Mg\text{-}O^-$ exist in the edge. Thus, amino acids are easily adsorbed on the edge through hydrogen bonding. The side chains of the amino acids in this work are relatively hydrophobic ones except for Asp and Glu. In addition, the surface of chrysotile generally has no charges. Based on these reasons, the side chain of amino acids should be placed on the surface of chrysotile so that this may cause a selective adsorption of L-amino acid on the edge of chrysotile.

Conclusively, the adsorption behavior of 10 amino acids by chrysotile was investigated, where chrysotile in the system of $MgO\text{-}SiO_2\text{-}H_2O$ had never been used for adsorption. Asp and Glu were more strongly adsorbed by chrysotile than the other amino acids. Amino acids examined in the present study except for Asp and Glu were not adsorbed strongly by chrysotile. There was a trend that amino acids possessing a large side chain were more strongly adsorbed than small amino acids except for Asp, Glu, Ile, and Ser. It was found that the strength of adsorption of amino acids by chrysotile was dominated by the dissociation of both amino acids and chrysotile, and also the solubility of amino acids into water. This trend of the adsorption of amino acids by chrysotile is similar to that by kaolinite [9]. This is probably due to the fact that the crystal structure of chrysotile is almost the same as that of kaolinite. Selectivity of D- or L-amino acids by chrysotile was not detected clearly in the present work. It was difficult to identify small peaks of the chromatogram for CD. The peaks were too small to distinguish whether the peaks indicate the selective adsorption of D- or L-amino acid by chrysotile or noises.

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