ADSORPTION OF TWENTY BIO-AMINO ACIDS BY NATURAL ALLOPHANE AND IMOGOLITE

Hashizume Hideo

National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
Fax: +81-29-860-4667
E-mail: HASHIZUME.Hideo@nims.go.jp

Abstract

Adsorption of 20 bio-amino acids at pH 4, 6, and 8 was carried out using short-range order soil minerals, viz. allophane and imogolite. The initial concentration of the amino acid solution was fixed at 2.5 mmol·dm^{-3}. We compared the extent of adsorption of the 20 amino acids by allophane and imogolite. Both allophane and imogolite adsorbed cysteine, aspartic acid, glutamic acid, lysine, arginine, and histidine considerably well at all three pH values, while they poorly adsorbed glycine, alanine, valine, leucine, isoleucine, methionine, threonine, tryptophan, and proline. The dissociation of each amino acid affects the adsorption behavior as well as the surface charges of allophane and imogolite. The amino acids that were easily adsorbed by allophone and imogolite harbored a carboxyl or amino group on their side chain. The extent of adsorption of allophane was quite similar to that of imogolite, because the surface charge of allophane is almost the same as that of imogolite at pH 4, 6, and 8. In addition, we investigated whether allophane and imogolite were able to differentiate between D- and L-amino acids. Our results suggest that such discrimination is not feasible.

Keywords: adsorption, amino acid, allophane, imogolite

1. Introduction

Interactions between clay minerals and organic molecules have been studied extensively, because clay minerals exhibit adsorptive and catalytic activities towards hydrophilic and hydrophobic organic molecules [1,2]. Clay minerals can differentiate between organic compounds because of their adsorptive activities. Certain clay minerals can polymerize or decompose organic molecules via their catalytic activities. A complex formed by the association of a clay mineral with an organic molecule can also exhibit new characteristics in addition to those displayed by the uncomplexed clay mineral [3]. Various characteristics of clay minerals might have played an important role during chemical evolution. These include accumulation of organic molecules through the process of adsorption and polymerization of organic molecules via their catalytic activities [4]. For example, montmorillonite prefers basic amino acids such as lysine, histidine, and arginine to acidic or non-polar amino acids [5]. Larger peptides are better adsorbed by montmorillonite or allophane than are smaller peptides or monomers [6,7]. Hedge and Hare showed that kaolinite could adsorb acidic and basic amino acids, and montmorillonite could adsorb basic amino acids but not acidic amino acids [8]. Hashizume showed that serpentine poorly adsorbed 10 different amino acids. These poorly adsorbed amino acids presumably existed on early earth, and the amount of serpentine-adsorbed amino acids was similar to that absorbed by kaolinite [8,9]. Acidic and basic amino acids would be similarly preferred by serpentine and kaolinite owing to the same crystal structures.

The clay minerals allophane and imogolite are obtained from the weathering of volcanic ash. Allophane is hollow and spherule-shaped, with a diameter of ~5 nm. Imogolite is tubular-shaped and has a diameter of ~2 nm. Its length varies from nanometers to several micrometers. The outer and inner layers of allophane and imogolite are composed of aluminum hydroxide (gibbsite structure) and silicate, respectively [10]. The Al/Si ratio is 1~2 for allophane and 2 for imogolite. Allophane and imogolite possess a surface charge. A positive, apparently neutral, or negative charge is under, about, or over pH 6, respectively [11]. They presumably existed on the early earth, and were most probably synthesized from volcanic ash with relative ease. They might have affected the syntheses and concentration as well as the polymerization of bio-organic molecules during the early stages of earth.

In order to gain an insight into the interactions between allophane/imogolite and various amino acids, we studied the allophane-/imogolite-mediated adsorption of 20 bio-amino acids at pH 4, 6, and 8. We also investigated the relationship between the adsorption of clay minerals and the type of amino acids involved, the dependence of adsorption on pH, and the difference between the adsorption mediated by allophane and imogolite.

2. Materials and methods

Allophane and imogolite were separated from the allophanic soil obtained from the Kitakami region in Iwate Prefecture, Japan. The isolation of allophane from the allophanic soil was carried out according to the method adopted by Hashizume and Theng [12]. Allophonic soil shows the presence of gelatinous imogolite, which was picked up and collected using tweezers. It was gently washed and dispersed in deionized water with pH 3.5~4 using an ultrasonic washer. The suspension was then removed to a volumetric cylinder. Particles under 2 μm (i.e., imogolite particles) were collected by gravitational sedimentation. After coagulating imogolite with 0.1 mol·dm^{-3} of NaCl, the pH was increased from 6.5 to 7.0. The coagulated solution was dialyzed using

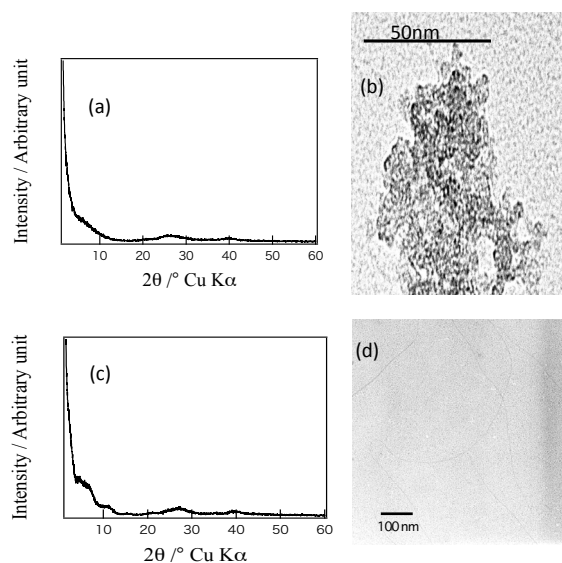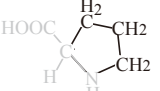Fig. 1 (a) XRD pattern of allophane. (b) TEM image of allophane. (c) XRD pattern of imogolite. (d) TEM image of imogolite.

deionized water, until all the salt was eliminated. The suspension was then dispersed in deionized water at an acidic pH (pH 3.5–4), again using an ultrasonic washer. The series of treatments performed to obtain imogolite was conducted twice to obtain pure imogolite. Before the dialysis, the suspension was treated with water containing 10% H_2O_2 at 100 °C to remove the natural organic matter. After the dialysis, the suspension was dried at 60 °C in an oven. Allophane and imogolite used were confirmed using XRD and TEM (Fig. 1). The XRD patterns (Fig. 1(a)) and TEM images (Fig. 1(b)) of allophane are quite similar to those reported by Hashizume and Theng [13]. The imogolite peaks in Fig. 1(c) are broader than the typical XRD peaks of imogolite reported in the Handbook of Clays and Clay Minerals [10], because we did not order the imogolite. The TEM image of imogolite shows the presence of long strings (Fig. 1 (d)). The 20 bio-amino acids used during this study have been listed in Table 1. All the amino acids purchased were of reagent grade. D- and L-glutamine were used in this study instead of DL-glutamine, because the latter could not be obtained.

The adsorption treatment was carried out at 25 ± 1 ºC. For the pH dependence, a 2.5 mmol·dm^{-3} amino acid solution was prepared by using solutions maintained at pH 2, 4, or 12, and pH-adjusted with 100 mmol·dm^{-3} of HCl and NaOH. After the

Table 1 Disociation constans and solubilities of 20 bio-amino acids.

Amino acid		side chain	Dissociation constant [15]				Solubility [16]	
			pK$_1$	pK$_2$	pK$_3$		(g/100g)	
			α-COOH	α-NH$_3^+$	side chain			
Glycine	(Gly)	H	2.35	9.78			20.0	
Alanine	(Ala)	CH$_3$	2.35	9.87			13.55	
Valine	(Val)	CH(CH$_3$)$_2$	2.29	9.74			6.38	
Leucine	(Leu)	CH$_2$CH(CH$_3$)$_2$	2.33	9.74			0.93	
Isileucine	(Ile)	CH(CH$_3$)CH$_2$CH$_3$	2.32	9.76			2.079	*
Phenyalanine	(Phe)	CH$_2$-⬡	2.16	9.18			1.290	*
Tyrosine	(Tyr)	CH$_2$-⬡-OH	2.20	9.11	10.13	(phenol)	0.0294	*
Serine	(Ser)	CH$_2$OH	2.19	9.21			4.11	*
Threonine	(Thr)	CH(OH)CH$_3$	2.09	9.10			9	*, L-, #
Cysteine	(Cyc)	CH$_2$SH	1.92	10.78	8.33	(Sulfhydryl)	0.1096	L-
Methionine	(Met)	CH$_2$CH$_2$SCH$_3$	2.13	9.28			2.908	*
Aspartic acid	(Asp)	CH$_2$COOH	1.99	9.90	3.90	(β-COOH)	0.629	*
Glutamic acid	(Glu)	CH$_2$CH$_2$COOH	2.00	9.47	4.07	(γ-COOH)	1.693	*
Aspargine	(Asn)	CH$_2$CO(NH$_2$)	2.1	8.84			2	*, L-, #
Glutamine	(Gln)	CH$_2$CH$_2$CO(NH$_2$)	2.17	9.13			2.5	?, L-, #
Lysine	(Lys)	CH$_2$CH$_2$CH$_2$CH$_2$NH$_2$	2.16	9.18	10.79	(ε-NH3+)	10	?, L-, #
Histidine	(His)	CH$_2$-⟨imidazole⟩	1.80	9.33	6.04	(imodazol)	5	?, L-, #
Arginine	(Arg)	CH$_2$CH$_2$CH$_2$NHC(NH)(NH$_2$)	1.82	8.99	12.48	(guanidine)	14.87	*, L-, #
Tryptophan	(Trp)	CH$_2$-⟨indole⟩	2.43	9.44			1.041	*, L-
Proline	(Pro)	⟨pyrrolidine ring⟩	2.95	10.65			5	?, L-, #

Solubility is measured at 25 C except * and ?. * is 20 C. ? is not shown. L- is an L-amino acid.
is reference [17] without reference [16]

adsorption treatment, the pH shifted from 2 to about 4, from 4 to about 6, and from 12 to about 8. A glass bottle with a stopper was used to mix and shake 60–100 mg of allophane and imogolite. In addition, 6 cm^3 of an amino acid solution was shaken for 40 h. The equilibrium pH was in the range 3.5–4.5, 5.5–6.5, or 8–9, for the solutions with an initial pH of 2, 4, and 12, respectively. After separating the supernatant from the suspension by centrifugation, the carbon contents of the supernatant and an initial solution were measured using a total organic carbon analyzer (Shimadzu TOC-5000A). The supernatant and the initial solution were analyzed eight times using TOC. We chose at least five analytical values out of eight in order to ensure a smaller CV value (i.e. the ratio of the standard deviation to the mean value). The concentrations of the supernatant and the initial solution were also determined. The extent of adsorption was estimated from the concentrations of the supernatant and the initial solution by obtaining their carbon contents, as shown in the equation below.

$$A = V \cdot (C_0 - C) / W \qquad (1)$$

where A, the adsorption; V, the volume added; C_0, the initial concentration of the adsorbate; C, the concentration of the adsorbate after the adsorption treatment; and W, the weight of the adsorbent. Experiments involving the adsorption of amino acids by allophane and imogolite were conducted more than twice. The extent of adsorption of the amino acids was reported as the mean value of several adsorption experiments.

3. Results and discussion
3. 1 Allophane

Figure 2 shows the extents of adsorption of the 20 bio-amino acids by allophane at pH 4, 6, and 8, respectively. Cys, Asp, Glu, Lys, and Arg were adsorbed more than the other amino acids. Adsorption was seen to a lesser extent in the case of Ala, Val, Leu, Ile, Phe, Tyr, Thr, Met, Trp, and Pro, than the other amino acids. The side-chains of the 20

bio-amino acids are shown in Table 1. The amino acids possessing alkyl groups (e.g., Ala, Val, Leu, and Ile) were not adsorbed well, and those possessing aromatic or cyclocarbon groups (e.g., Phe, Tyr, Trp, and Pro) were not preferred by allophane. Pro behaved similarly to the amino acids possessing an alkyl chain. The sulfur-containing side-chain of Met showed no effect on adsorption. Therefore, the adsorption of Met was similar to that of other amino acids possessing alkyl groups. Asp and Glu contain a negatively charged carboxyl group in their side-chain, whereas Lys and Arg contain a positively charged amino group in their side-chain. Allophane shows a positive or negative charge at various pH values, and adsorbs Asp, Glu, Lys, and Arg to a significant extent. Ser and Thr contain a hydroxide group. Asn changes the hydroxide group in the carboxyl side chain of Asp to an acid amide (β-monoamide). Similarly, Gln is the γ-amide of Glu. The side-chains of Ser, Thr, Asn, and Gln are uncharged and polar, and their polarity does show some effect on allophane adsorption. Gly contains a hydrogen atom in the side-chain, whereas His contains an imidazole group in its side- chain. His is similar in nature to Lys and Arg, but the adsorption of His is not greater than that of Lys or Arg. The cyclic structure of the imidazole group probably affects the adsorption of His. The adsorption of Gly is similar to that of the amino acids containing an alkyl side-chain.

We showed that the adsorption varied at various pH values. The dissociation constant of each amino acid is mentioned in Table 1. When the pH changed from a lower to a higher value, the charge on each amino acid and that on the surface of allophane change according to the pH. At pH 4, allophane shows a positive charge. Asp and Glu were adsorbed better than the other amino acids. Because Asp and Glu contained negatively charged –COO$^-$ groups instead of the neutral –COOH groups, the positively charged allophane could easily adsorb them. The adsorption of Asn or Gln was slightly greater than that of all other amino acids, except Cys, Asp, and Glu. The
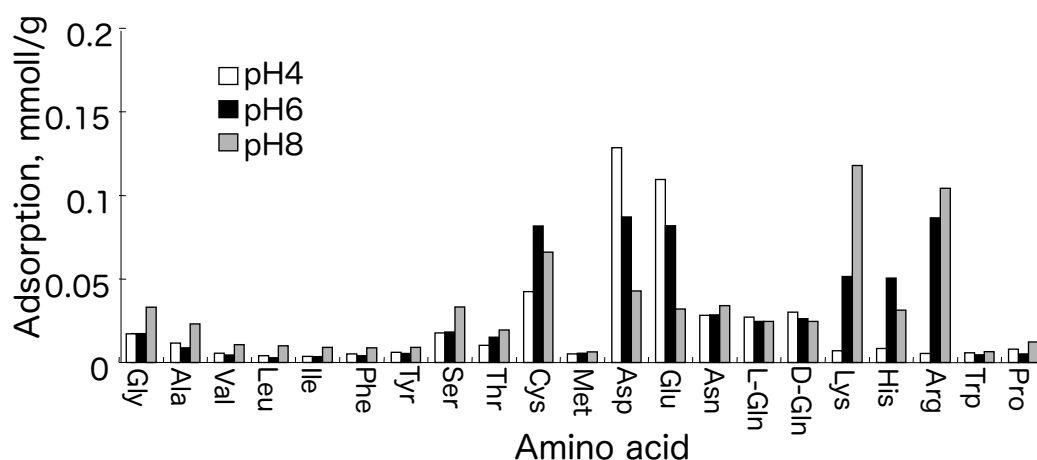

Fig. 2 Adsorption of 20 amino acids by allophane at pH 4, 6, and 8.

polarity of the side chains of Asn and Gln might have had some effects on its adsorption behavior. Most amino acid besides Asp, Glu, Asn, and Gln contain charged –COO⁻ and –NH³⁺ groups in their α-amino acid structures. These amino acids were not adsorbed well by allophane, because the positive charge on the amino groups of these amino acids repelled the positive surface charge of allophane. At pH 6, the allophane surface is neutrally charged. As the negative charge of allophane increased, the adsorption sites of the two –COO⁻ groups of Asp and Glu showed a corresponding decrease owing to the repulsion between two negative charges. The amino group of Lys and the guanidine group of Arg are positively charged, whereas the imidazole group of the side chain of His is neutral or shows a slight positive charge. Lys, Arg, and His were adsorbed better by allophane, because of the increase in the negative charge of allophane. At pH 8, the amino acids containing hydrogen or alkyl group side-chains (e.g., Gly, Ala, Val, Leu, and Ile) were adsorbed slightly better than the same amino acids at pH 4 and 6, respectively. Because the allophane surface is negatively charged and because the amino groups of certain amino acids are positively charged, such positively charged amino acids were adsorbed well by allophane. The adsorption of Phe, Tyr, Ser, and Thr could be explained using the same reasoning. In the case Asp and Glu, the extent of adsorption decreased, because the allophane surface was negatively charged and because the two carboxyl groups on Asp and Glu also carried a negative charge. The amino groups of Asp and Glu are either neutral or carry a slight positive charge. Because the amino groups of Lys and Arg are positively charged, and because the allophane surface shows a negative charge, Lys and Arg were easily adsorbed by allophane. The imidazole group of His being neural in nature, the extent of adsorption of His decreased at pH 8, as compared to its adsorption at pH 4 and 6, respectively.

Cys was adsorbed well by allophane at a pH of 4, 6, and 8, respectively. The adsorption of Cys hardly showed an effect on the corresponding dissociation constants at various pH values. Greathouse et al. investigated the adsorption mechanism of methyl, ethyl, and n-propyl thiols on $Al(OH)_3$, $FeO(OH)$, and carbon surfaces [14]. They showed that the hydrogen and sulfur atoms of the thiol bonded with an oxygen and a hydrogen of $Al(OH)_3$ via van der Waals interactions. The outer surface of allophane is covered with aluminum hydroxide. A sulfur and a hydrogen atom of the sulfhydryl group of Cys might possibly bind with the hydrogen and oxygen atoms of the aluminum hydroxide present on the allophane surface. Cys is adsorbed well by allophane regardless of the pH.

3.2 Imogolite

The adsorption of 20 bio-amino acids by imogolite is shown in Fig. 3. The surface charge of imogolite is positive, almost neutral, and negative at pH 4, 6, and 8, respectively. The change of the surface charge of imogolite is almost similar to that of allophane.

The adsorption of amino acids on the imogolite surface is similar to that on the allophane surface. The adsorption of Cys, Asp, Glu, Lys, His, and Arg on the imogolite surface is higher than that of any other amino acids on the same surface. The amino acids containing alkyl, aromatic, and heterocyclic groups in the side-chain are poorly adsorbed by imogolite. The pH dependence of the adsorption tendency of imogolite is similar to that of allophane. In general, the adsorption by the imogolite surface was higher than that by the allophane surface, as evident from the comparison of Fig. 3 with Fig. 2.

3.3 Solubility

Solubility affects the adsorption behavior as well as the kinetics of dissociation. Generally, materials showing high solubility are not adsorbed well by the adsorbent, and materials showing low solubility are adsorbed well by the adsorbent. For example, the adsorption from Gly to Tyr in Figs. 2 and 3 is inversely correlated with the solubilities of these amino acids mentioned in Table 1. The adsorption of Lys, His, and Arg is not strongly correlated with their corresponding solubilities, presumably because their
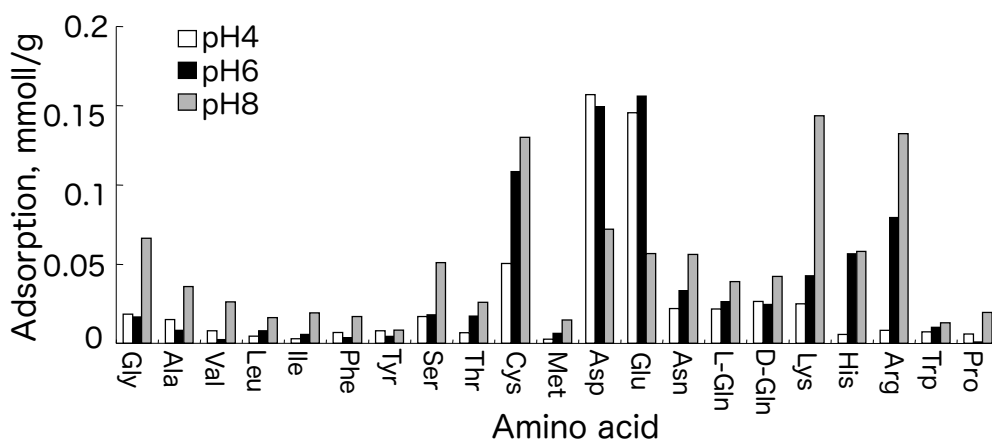

Fig. 3 Adsorption of 20 amino acids by imogolite at pH 4, 6, and 8.

large positive charges completely outweigh the effect of their corresponding solubilities. The adsorption of Lys, His, and Arg, as expected from their solubilities, is in the order His > Lys > Arg. However, Figs. 2 and 3 show a slightly different order (Lys > Arg > His). At pH 4, the extent of adsorption of these amino acids on the allophane surface was nicely correlated with their corresponding solubilities (i.e., His > Lys > Arg).

The neutral amino acids showed a good inverse correlation with their corresponding solubilities.

4. Summary

The adsorption of 20 bio-amino acids by allophane and imogolite was studied at a concentration of 2.5 m mol·dm^{-3} of the initial solutions (Figs. 2 and 3). The extent of adsorption varied according to the pH. In other words, it depended on the dissociation of the amino acid and the corresponding surface charge on allophane or imogolite at that particular pH. The surface charge of allophane is quite similar to that of imogolite. The tendency of adsorption of various amino acids on the allophane surface is therefore similar to that on the imogolite surface. The amino acids with methyl or heterocyclic group side-chains were not adsorbed well by allophane and imogolite. Asp and Glu, with a carboxyl group in the side-chain, and Lys, His, and Arg with amino or imidazole groups in the side-chain were adsorbed well by allophane and imogolite.

We also investigated the ability of allophane and imogolite to discriminate between D- and L-amino acids. The supernatants were analyzed using an HPLC instrument attached with a CD-detector. However, we could not detect a clear difference the between D- and L-amino acids on the chromatograph.

Hedge and Hare studied the adsorption of sixteen amino acids on kaolinite and montmorillontie surfaces [8]. Asp and Lys were adsorbed well by kaolinite. Montmorillonite adsorbed Lys well, but adsorbed Asp poorly. Thr amino acids with alkyl group side-chains were not adsorbed by montmorillonite and kaolinite. These results are quite similar to the results obtained by us. The structure of kaolinite reveals a stratification of the aluminum hydroxide and silicate sheets. Allophane and imogolite contain only one layer that stratifies the aluminum hydroxide and silicate sheets. Because of the similarities in the crystal structures of kaolinite, allophane, and imogolite, the adsorption shown by allophane and imogolite would be similar to that shown by kaolinite.

5. Acknowledgement

References

1. Hashizume, H., Gaast, S., and Theng, B. K. G., Interactions of Clay Minerals with RNA Components, pp. 61-80, "Evolutionary Biology: Exobiology and Evolutionary Mechanisms," Pontarotti, P. (ed.) Springer Heidelberg New York Dordrecht London (2013)
2. Hashizume, H., Adsorption of Aromatic Compounds in Water by Talc, Clay Science, 14, 61-64, (2009).
3. Tamura, K., and Morimoto, K., Synthesis of Brucite-Sugar Alcohol Hybrids, J. Soc. Inorganic Materials, Japan 20, 414-417 (2013) (in Japanese)
4. Hashizume, H., Role of Clay Minerals in Chemical Evolution and Origin of Life, pp. 191-208, "Clay Minerals in Nature," Valaskova, M., and Marthynakova, G. S. (eds.) INTECH, Croatia (2012)
5. Greenland, D. J., Laby, R. H., and Quirk, J. P., Adsorption of Amino-acids and Peptides by Montmorillonite and Illite, Trans. Farad. Soc., 61, 2024-2035, (1965)
6. Greenland, D. J., Laby, R. H., and Quirk, J. P., Adsorption of Glycine and its Di-, Tri-, and Tetra-Peptides by Montmorillonite, Trans. Farad. Soc., 58, 829-841, (1962)
7. Hashizume, H., and Theng, B. K. G., Adsorption of L-alanine, Monomer, Dimer, Trimer, Tetramer and Pentamer by Some Allophanes, pp. 105-107, "Water-Rock Interaction," Arehart, G. B., and Hulston, J. R. (eds.) Proc. The 9th Inter. Symp. Water-Rock Interaction, A.A Balkema, Rotterdam/Broolfield, (1998)
8. Hedge, J. I., and Hare, P. E., Amino Acid Adsorption of Clay Minerals in Distilled Water, Geochim. Cosmochm. Acta, 51, 255-259, (1987).
9. Hashizume, H., Adsorption of Some Amino Acids by Chrysotile, Viva Origino, 35, 60-65, (2007).
10. The Clay Science Society of Japan ed., Handbook of Clays and Clay Minerals (the 3rd edition), Gihoudou, pp. 88-92, (2009)
11. Theng, B. K. G., Russell, M., Churchman, G. J., and Parfitt, R. L., Surface properties of Allophane, Halloysite and Imogolite, Clays Clay Miner., 30, 143-149, (1982).
12. Hashizume, H., and Theng, B. K. G., Adsorption of DL-alanine by Allophane: Effect of pH and Unit Particle Aggregation, Clay Miner., 34, 223-238 (1999).
13. Hashizume, H., and Theng, B. K. G., Adenine, Adenosine, Ribose and 5′ -AMP Adsorption to Allophane, Clays Clay Miner., 55, 599-605, (2007).
14. Greathouse, J. A., Hart, D. B., and Ochs, M. E., Alcohol and Thiol Adsorption on (Oxy)hydroxide and Carbon Surfaces: Molecular Dynamics Simulation and Desorption Experiments, J. Phys. Chem. C, 116, 26756-26764, (2012).
15. Voet, D., and Voet, J. G., Biochemistry, John Wiley and Sons, New York, (1990). (translated into Japanese by Tamiya, N., Matsumura, M., Yagi, T., and Yoshida, H., Voet Seikagaku (Jo), Tokyokagakudoujin, pp. 48-59 (1992).
16. Chemical Society of Japan ed., Kagakubinran Kisohen II. (the 4th edition) Tokyo Maruzen Shuppan, pp. II157-II189, (1995).
17. Chemical book (www. Chemicalbook.com) Accessed 7th May (2014).