

Amino Acid Interaction with and Adsorption on Clays: FT-IR and Mössbauer Spectroscopy and X-ray Diffractometry Investigations

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Abstract In the present paper, the adsorption of amino acids (Ala, Met, Gln, Cys, Asp, Lys, His) on clays (bentonite, kaolinite) was studied at different pH (3.00, 6.00, 8.00). The amino acids were dissolved in seawater, which contains the major elements. There were two main findings in this study. First, amino acids with a charged R group (Asp, Lys, His) and Cys were adsorbed on clays more than Ala, Met and Gln (uncharged R groups). However, 74% of the amino acids in the proteins of modern organisms have uncharged R groups. These results raise some questions about the role of minerals in providing a prebiotic concentration mechanism for amino acids. Several mechanisms are also discussed that could produce peptide with a greater proportion of amino acids with uncharged R groups. Second, Cys could play an important role in prebiotic chemistry besides participating in the structure of peptides/proteins. The FT-IR spectra showed that the adsorption of amino acids on the clays occurs through the amine group. However, the Cys/clay interaction occurs through the sulfhydryl and amine groups. X-ray diffractometry showed that pH affects the bentonite interlayer, and at pH 3.00 the expansion of Cys/bentonite was greater than that of the samples of ethylene glycol/bentonite saturated with Mg. The Mössbauer spectrum for the sample with absorbed Cys showed a large increase (~20%) in ferrous ions. This means that Cys was able to partially

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reduce iron present in bentonite. This result is similar to that which occurs with aconitase where the ferric ions are reduced to Fe 2.5.

Keywords Amino acids · Adsorption · Clays · FT-IR spectroscopy · Mössbauer spectroscopy · X-ray diffractometry

Introduction

Since Bernal (1951) first suggested that mineral clays could have played an important role in the origin of life, because they took part in processes such as the selection and concentration of monomers from dilute solution and their subsequent condensation to form biopolymers, several studies have been undertaken to examine this possibility. It should be pointed out that the study of the formation of amino acids and subsequent condensation to form peptides/proteins is important for prebiotic chemistry since the major reactions of today's living beings involve amino acids/peptides/proteins (Darnell et al. 1990).

Numerous papers about the adsorption of amino acids on minerals/clays/sediments have been published such as: Ala/Gly/Ser/Phe/His on β -FeOOH.Cl_n (Holm et al. 1983); several amino acids on clays (Paecht-Horowitz 1978; Aufdenkampe et al. 2001; Ding and Henrichs 2002); Lys/Leu/Asp on titanium dioxide (Rogacheva and Bobyrenko 1985); amino acids on hydroxyapatite/calcite/albite (Tanaka et al. 1989; Churchill et al. 2004); Gly/Lys/Glu on hematite (Ben-Taleb et al. 1994); Gly/Ser/Cys on pyrite (Bebié and Schoonen 2000); pool of 20 protein amino acids on silica gel/poly A (Mellersh and Wilkinson 2000); various amino acids on several river or marine sediments (Henrichs and Sugai 1993; Aufdenkampe et al. 2001; Montluçon and Lee 2001; Ding and Henrichs 2002) and several amino acids on silica/sand/quartz (Basiuk and Gromovoy 1996; Basiuk 2002; Zaia et al. 2002; Churchill et al. 2004). In general, all these papers showed that amino acids with positively or negatively charged R groups were adsorbed more than other amino acids.

A study published by Klapper (1977) on the average occurrence of amino acids in over 200 proteins showed that 74% of the amino acids of today's proteins are amino acids with non-charged R groups. However, based on the results shown above, it would be expected that peptides would contain much more amino acids with charged R groups than uncharged R groups, since minerals adsorb much more amino acids with negatively or positively charged R groups. As discussed by Zaia et al. (2002), Zaia (2004) and Zaia and Zaia (2006) these results raise the following questions about the role of minerals in providing a concentration mechanism for amino acids in prebiotic chemistry. What were the mechanisms involved in producing peptides/proteins with more amino acids with uncharged R groups than those with charged R groups and Cys? Should we expect the types of amino acids that are adsorbed on minerals to reflect the composition of present-day proteins? Was the adsorption of amino acids on minerals important in the origin of life? Zaia (2004) and Zaia and Zaia (2006) pointed out several mechanisms that could be involved in producing peptides/proteins composed of more amino acids with uncharged R groups than of those with charged R groups, such as: adsorption on liposome membranes (Böhler et al. 1996; Blocher et al. 1999, 2000; Luisi et al. 2000); adsorption of amino acids by purine bases preadsorbed on bare graphite (Sowerby et al. 2002) and wet/dry cycles (Yanagawa et al. 1990; Suwannachot and Rode 1999; Plankensteiner et al. 2002). Andersson and Holm (2000) studied the stability of Asp, Ser, Leu and Ala under buffered and non-buffered hydrothermal conditions (200°C, 50 bar). Among these four amino acids, Ala was the most stable one, where the half-life of Ala in the non-buffered control experiment was 27 h and

in the buffered experiment 380 h. They also observed that in the early stage of the experiments, the concentration of Ala increased probably because of the dehydration of serine, which was also responsible for the formation of Gly, which was not present initially.

However, as pointed out by Zaia (2004), many of the studies of amino acid adsorption were carried out under conditions far from naturally occurring ones. It also is important to understand how amino acids are adsorbed on clays since this could be used to propose a mechanism for the formation of peptide bonds (White et al. 1984; Bujdák and Rode 2001), as well as to better understand several mechanisms involved in the metalloproteins of modern organisms (Cowan 1993).

The present paper describes the adsorption of amino acids (Ala, Met, Gln, Cys, Asp, Lys, His) on clays (bentonite, kaolinite) at different pH (3.00, 6.00 and 8.00). The amino acids were dissolved in artificial seawater, which contain the major elements; it should be pointed out that as far as we know there are no papers describing the adsorption of amino acids under conditions so close to a naturally occurring one as well as using a large variety of amino acids and wider range of pH. The present paper also shows a study of the interaction of amino acids and clays using FT-IR and Mössbauer spectroscopies and X-ray diffractometry. It also should be pointed out that as far as we know there are no papers describing the interaction of amino acids and clays using all the methods that we used in this study.

Materials and Methods

Materials

All reagents were of analytical grade.

Clays

Bentonite was purchased from Acros Organics – NJ, USA. Kaolinite was a gift of Dr Carlos H. Sampaio of LAPROM/CT-UFRGS-RS, Brazil. All clays were used without further preparation.

Bentonite: *chemical composition*: $\text{SiO}_2=73.0\%$, $\text{Al}_2\text{O}_3=14.0\%$, $\text{Fe}_2\text{O}_3=2.7\%$, $\text{CaO}=0.2\%$, $\text{MgO}=1.1\%$, $\text{Na}_2\text{O}=0.6\%$, $\text{K}_2\text{O}=1.9\%$; *surface area*=240 m^2/g (Acros Organics). Kaolinite: *chemical composition*: $\text{SiO}_2=45.7\%$, $\text{Al}_2\text{O}_3=38.9\%$, $\text{Fe}_2\text{O}_3=0.3\%$, $\text{CaO}=0.1\%$, $\text{MgO}=0.1\%$, $\text{Na}_2\text{O}=0.1\%$, $\text{K}_2\text{O}=0.6\%$, $\text{TiO}_2=0.2\%$; *surface area*=20 m^2/g (from LAPROM/CT-UFRGS).

Amino Acids

All L-amino acids were from Synth (Brazil) or Nuclear (Brazil) and were used as received. Amino acid abbreviations are given according to the recommendations of the IUPAC-IUB commission on biochemical nomenclature: Ala, alanine; Met, methionine; Gln, glutamine; Cys, cysteine; Asp, aspartic acid; Lys, lysine; and His, histidine.

Seawater

The following substances were weighed and dissolved in 1.0 l of distilled water: 28.57 g of sodium chloride, 3.88 g of magnesium chloride, 1.787 g of magnesium sulfate, 1.308 g of

calcium sulfate, 0.832 g of potassium sulfate, 0.124 g of calcium carbonate, 0.103 g of potassium bromide and 0.0282 g of boric acid.

Sample Preparation

The amino acids (Ala, Met, Gln, Cys, Asp, Lys, or His) were dissolved in seawater at a concentration of $240 \mu\text{g ml}^{-1}$ and as a saturated solution. Each clay (Bentonite, Kaolinite) was processed as follows: to three different sets of four tubes (15 ml) containing 500 mg of clay (Bentonite or Kaolinite) were added: (a) 5.00 ml of seawater, (b) 5.00 ml of seawater with $240 \mu\text{g ml}^{-1}$ of amino acid (Ala, Met, Gln, Cys, Asp, Lys, or His) and (c) 5.00 ml of seawater with saturated amino acid (Ala, Met, Gln, Cys, Asp, Lys, or His). The pH was adjusted to 3.00, 6.00 or 8.00 with HCl or NaOH. The tubes were mixed for 24 h, after which they were spun for 15 min at 2,000 rpm; the aqueous phase was used for the amino acid analysis and the solid was dried in an oven at 40°C for 24 h and used for the FT-IR and Mössbauer spectroscopy and X-ray diffractometry.

Methods

Visible Spectrophotometric Methods

Absorbance was read with a spectrophotometer, Spectronic Genesys. The amino acids (Ala, Met, Gln, Asp, Lys, His) were determined using the ninhydrin method as described by Fisher et al. (1963) and Cys was determined using *p*-benzoquinone as described by Zaia et al. (1999). The following equation was used for the calculation of the amount of amino acid adsorbed on 500 mg of clay (bentonite, kaolinite). Adsorbed AA/ μg = $[1,200 - (1,200 \times \text{Abs of solution after mixing for 24 h with clay/Abs of sample of } 240 \mu\text{g ml}^{-1} \text{ of amino acid})]$.

Infrared Spectrophotometric Method

The IR spectra were recorded with a FT-IR 8300 Shimadzu using pressed KBr disks and a spectral resolution of 4 cm^{-1} , and each spectrum was obtained after acquiring 120 spectra. FT-IR analysis was carried out with clay samples with and without amino acid adsorption. About 10 mg of clay samples plus 200 mg of KBr were weighed and ground in an agate mortar with a pestle until a homogeneous mixture was obtained. Disc pellets were prepared and spectra were recorded from 400 to $4,000 \text{ cm}^{-1}$. FT-IR spectra were analyzed by the Origin program (5.0, 2001).

Mössbauer Spectroscopy (MS) Method

Mössbauer spectroscopy (MS) characterizations were performed in transmission geometry, using a conventional Mössbauer spectrometer, in a constant acceleration mode. The X-rays were provided by a $^{57}\text{Co(Rh)}$ source. The Mössbauer spectra were analyzed with a non-linear least-square routine, with Lorentzian line shapes. All isomer shift (IS) data given are relative to $\alpha\text{-Fe}$ throughout this paper.

X-ray Diffractometry

The X-ray diffractograms were obtained in a D-6000 Shimadzu, using $\text{Cu K}\alpha$ radiation with Ni filter and the scanning parameters were set at $0.02^\circ 2\theta$ step width, count time 0.6 s

and a measurement range from 2 to 30° 2 θ . The samples (powder) were placed on a glass plate and few drops of deionized water were added, after which another glass plate was placed on top, and the samples were dried at 25°C. X-ray diffractometry was analyzed by the software Grams/386 v 4.0 (Galactic Ind. Corp.).

Statistical Analysis

Comparisons between means were assessed by using: ANOVA and Student-Newman-Keuls test (S.N.K. test) at a significance level of $p < 0.05$.

Results and Discussion

Tables 1 and 2 show the adsorption of amino acids (Ala, Met, Gln, Cys, Asp, Lys, His) dissolved in seawater (see methodology) at different pH (3.00, 6.00 and 8.00) on kaolinite

Table 1 Amount in micrograms of amino acids adsorbed on 500 mg of kaolinite in several pHs

R group	Amino acid ^a	pH at time $t=0^b$	Range of pH ^c	Adsorbed amount (μg)
Non-polar aliphatic	Alanine	3.00	2.56–3.45	192.3 \pm 21.8 (6)A
		6.00	5.45–7.02	163.3 \pm 10.6 (4)
		8.00	7.70–8.41	243.2 \pm 31.7 (7) β , γ , π , θ
Polar uncharged	Methionine	3.00	2.77–3.08	149.3 \pm 22.7 (6)a, B, C
		6.00	5.70–6.71	31.7 \pm 13.3 (7)a
		8.00	8.00–8.52	235.7 \pm 17.1a, β , γ , π , χ
	Glutamine	3.00	3.17–3.32	494.3 \pm 62.2 (4)b, G
		6.00	5.99–6.39	220.8 \pm 16.6 (4)a, ∇
		8.00	7.86–8.54	397.1 \pm 48.7 (4)b, α
	Cysteine	3.00	2.67–3.11	1109.8 \pm 69.9 (4)
		6.00	6.53–6.72	1154.6 \pm 19.9 (5)
		8.00	8.06–8.34	1193.4 \pm 3.6 (5)
Negatively charged	Aspartic Acid	3.00	2.45–3.35	429.3 \pm 31.9 (3)H
		6.00	6.53–6.72	268.5 \pm 13.4 (5)
		8.00	7.80–8.25	321.5 \pm 56.5 (8) β , ϕ
Positively charged	Lysine	3.00	2.82–3.07	219.1 \pm 46.1 (5)b, B, D, E
		6.00	5.32–5.63	453.9 \pm 19.7 (4)a
		8.00	7.86–8.26	318.0 \pm 13.4 (4)b, β , γ , μ
	Histidine	3.00	2.89–3.38	207.0 \pm 41.6 (7)a, B, D, F
		6.00	6.35–7.05	531.2 \pm 45.2 (5)a
		8.00	8.05–8.42	893.7 \pm 78.0 (7)a

The results are presented as mean \pm standard error of mean. The number of sets is given in parentheses with four samples each set. SNK test ($p < 0.05$) for Ala ANOVA test ($F=2.19$, $P=0.149$); for Met ANOVA test ($F=32.79$, $P=0.000$), SNK test a/a; for Gln ANOVA test ($F=8.85$, $P=0.007$), SNK test a/b; for Cys ANOVA test ($F=1.28$, $P=0.317$); for Asp ANOVA test ($F=1.68$, $P=0.225$); for Lys ANOVA test ($F=12.43$, $P=0.002$), SNK test a/b; for His ANOVA test ($F=35.80$, $P=0.000$), SNK test a/a. For all amino acids at range of pH 3.00 ANOVA test ($F=55.16$, $P=0.000$) and SNK test ($p > 0.05$) A/B, C/D, E/F and G/H; for all amino acids at range of pH 6.00 ANOVA test ($F=282.02$, $P=0.000$) and SNK test ($p > 0.05$) ∇ /; for all amino acids at range of pH 8.00 ANOVA test ($F=50.61$, $P=0.000$) and SNK test ($p > 0.05$) α / β , ϕ / γ , μ / π and θ / χ .

^a Amino acids (1,200; 0 μg /5.0 ml) were dissolved in seawater as described in “Materials and methods.”

^b pH adjusted at time $t=0$

^c The ranges of pH after the samples were tumbled for 24 h

Table 2 Amount in micrograms of amino acids adsorbed on 500 mg of bentonite in several pHs

R group	Amino acid ^a	pH at time $t=0^b$	Range of pH ^c	Adsorbed amount (μg)
Non-polar aliphatic	Alanine	3.00	2.48–3.21	245.1±35.0 (5)b, E
		6.00	6.04–6.53	147.4±13.9 (5)a
		8.00	7.50–8.50	260.6±22.4 (6)b, α
Polar uncharged	Methionine	3.00	2.73–3.12	521.3±59.4 (7)a, B, C
		6.00	5.90–6.69	293.1±32.7 (6)a, ∇
		8.00	8.15–8.48	672.9±43.4 (5)a
	Glutamine	3.00	2.79–3.82	266.1±11.3 (6)b, F
		6.00	6.33–6.83	321.1±42.6 (7)b
		8.00	7.84–8.33	508.6±22.5 (4)a, φ
	Cysteine	3.00	2.81–2.93	1185.8±14.2 (3)
		6.00	6.00–6.22	1155.7±10.6(5)
		8.00	8.24–8.42	1173.5±16.0 (5)
Negatively charged	Aspartic Acid	3.00	2.65–2.96	607.2±38.6 (4)A
		6.00	6.55–6.95	468.2±42.6 (4)
		8.00	7.95–8.25	477.2±44.8 (6)γ
Positively charged	Lysine	3.00	2.83–2.89	785.4±39.4 (6)b
		6.00	6.44–6.95	806.0±35.5 (5)b
		8.00	8.00–8.32	1007.4±12.2 (4)a
	Histidine	3.00	2.46–3.37	551.6±41.0 (5)a, B, D
		6.00	6.74–7.94	928.6±42.9 (6)a
		8.00	8.02–8.32	273.1±21.5 (5)a, β

The results are presented as mean ± standard error of mean. The number of sets is given in parentheses with four samples each set. SNK test ($p < 0.05$) for Ala ANOVA test ($F = 5.90$, $P = 0.015$), SNK test a/b; for Met ANOVA test ($F = 13.99$, $P = 0.000$), SNK test a/a; for Gln ANOVA test ($F = 12.06$, $P = 0.000$), SNK test a/b; for Cys ANOVA test ($F = 1.07$, $P = 0.380$); for Asp ANOVA test ($F = 2.82$, $P = 0.103$); for Lys ANOVA test ($F = 10.91$, $P = 0.002$), SNK test a/b; for His ANOVA test ($F = 79.09$, $P = 0.000$), SNK test a/a. For all amino acids at range of pH 3.00 ANOVA test ($F = 44.02$, $P = 0.000$) and SNK ($p > 0.05$) test A/B, C/D, and E/F; for all amino acids at range of pH 6.00 ANOVA test ($F = 109.49$, $P = 0.000$) and SNK test ($p > 0.05$) ∇/; for all amino acids at range of pH 8.00 ANOVA test ($F = 127.0$, $P = 0.000$) and SNK ($p > 0.05$) test α/β and φ/γ.

^a Amino acids (1,200;0 μg/5.0 ml) were dissolved in seawater as described in “Materials and methods.”

^b pH adjusted at time $t = 0$

^c The ranges of pH after the samples were tumbled for 24 h

and bentonite, respectively. It should be pointed out that in the literature there are several papers about the adsorption of amino acids on minerals, but as far as we know there is no paper using conditions so close to a naturally occurring condition as used in this study. The adsorption of ATP on pyrite was influenced by divalent (Mg^{2+} and Ca^{2+}) cations from seawater (Tessis et al. 1999), and the adsorption of nucleic acids on clays (montmorillonite and kaolinite) was influenced by monovalent (Na^+) and divalent (Mg^{2+} and Ca^{2+}) cations (Franchi et al. 2003). Thus, it is important to study the effect of seawater on the adsorption of amino acids. The seawater used in these experiments contains all the major elements, and we also used a wide range of pH (3.00–8.00). As reviewed by Holm and Andersson (2005) and Holm et al. (2006), this range of pH could even be wider in hydrothermal environments.

The amount of Ala (non-polar aliphatic R group), Cys (polar, uncharged R group) and Asp (negatively charged R group) adsorbed on kaolinite as well as of Cys (polar uncharged R group) and Asp (negatively charged R group) adsorbed on bentonite did not change in the pH range studied here (Tables 1 and 2; S.N.K. > 0.05). The adsorption of Asp (negatively charged R group) on kaolinite and bentonite did not change with variation in

pH, which could be due to: isoelectric point ($pI=2.77$), $pK(-COOH)=1.88$, $pK-(NH_3^+)=9.60$ are out of range of pH studied here (Lehninger et al. 1993). As determined by De Santana et al. (2006), the point of zero charge ($pHpzc$) for bentonite and kaolinite are 1.88 and 4.52, respectively. Although the $pHpzc$ for kaolinite would appear to be in the pH range studied in this paper, it should be pointed out that determination of $pHpzc$ depends on the method used, and for kaolinite this value varies from 2.7 to 4.5 (Parks 1967; Appel et al. 2003; De Santana et al. 2006). Thus, the $pHpzc$ for both clays could be out of the pH range used in this study. It also should be pointed out that the pK (R group) for Asp is 3.65 and that for both clays the adsorption of Asp at pH 3.00 was little more than at other pH (Tables 1 and 2). Thus, no variation in adsorption of Asp on clays (kaolinite, bentonite) could be due to pH (3.00–8.00); the surface charge (negative net charge) of the clays did not change and Asp remained completely ionized ($^-OOC(CH_2)(H)C_\infty(NH_3^+)COO^-$). Rogacheva and Bobyrenko (1985) studied the adsorption of several amino acids including Asp (negatively charged R group) on titanium dioxide and observed that adsorption decreased with an increase in pH. However, Ben-Taleb et al. (1994) showed that the adsorption of Glu (negatively charged R group) on hematite was maximal at a pH close to the pI of Glu. The amount of Cys (polar uncharged R group) adsorbed on kaolinite and bentonite did not change with variation in pH, probably because of the higher adsorption capacity of minerals for this amino acid. Bebié and Schoonen (2000) observed a very strong interaction between Cys and pyrite, possibly through its thiol group with sulfur-deficient (iron rich) surface sites. As pointed out by Basiuk (2002), the sulfide group of Cys noticeably decreases ΔG° . Therefore, its adsorption will be increased. There is no simple explanation for the lack of variation in the adsorption of Ala (non-polar aliphatic R group) on kaolinite with pH (Table 1), since on bentonite its adsorption varied with pH (Table 2). Tanaka et al. (1989) also showed that the adsorption of Ala on hydroxyapatite was almost independent of the solution pH. Grzegorzczuk and Carta (1996) studied the adsorption of several amino acids (Leu, non-polar aliphatic R group; Phe, aromatic R group; and Try, aromatic R group) on a range of porous polymeric adsorbents and showed that adsorption isotherms are essentially independent of pH. Bebié and Schoonen (2000) also showed that the interaction Ala/pyrite depends on the pH and the Fe^{2+} concentration of the solution.

The adsorption of Met and Gln, both amino acids with polar uncharged R groups, on kaolinite and Met (polar uncharged R group) and Ala (non-polar aliphatic R group) on bentonite at pH 6.00 was lower than at other pH levels (pH 3.00 and 8.00; S.N.K.<0.05; Tables 1 and 2). The adsorption of Gln (polar uncharged R group) on bentonite at pH 8.00 was greater than at other pH (3.00 and 6.00; S.N.K.<0.05; Table 2). Holm et al. (1983) observed that the adsorption of Ala (non-polar aliphatic R group), Gly (non-polar aliphatic R group) and Ser (polar uncharged R group) on β -FeOOH. Cl_n increased at higher pH. Rogacheva and Bobyrenko (1985) showed that adsorption of Leu (non-polar aliphatic R group) on titanium dioxide decreased with increased pH. On the other hand, Ben-Taleb et al. (1994), Kalra et al. (2000) and Basiuk (2002) found that the maximum adsorption of Ala as well as other amino acids on hematite (Ala, Gly, Lys, Thr, Glu), montmorillonite (Ala, Gly) and bare silica occurred close to their pI . Meng et al. (2004) also observed that the adsorption of Gly (non-polar aliphatic R group) on silica was maximum at pH 6.00 (Gly, pI 5.97; Lehninger et al. 1993).

The adsorption of Lys (positively charged R group) on kaolinite at pH 6.00 was greater than at other pH (3.00 and 8.00; S.N.K.<0.05; Table 1). Conversely, the adsorption of Lys on bentonite at pH 8.00 was greater than at other pH (3.00 and 6.00; S.N.K.<0.05; Table 2). The adsorption of His (positively charged R group) on kaolinite increased at higher pH (S.N.K.<0.05; Table 1). However, the adsorption of His on bentonite increased

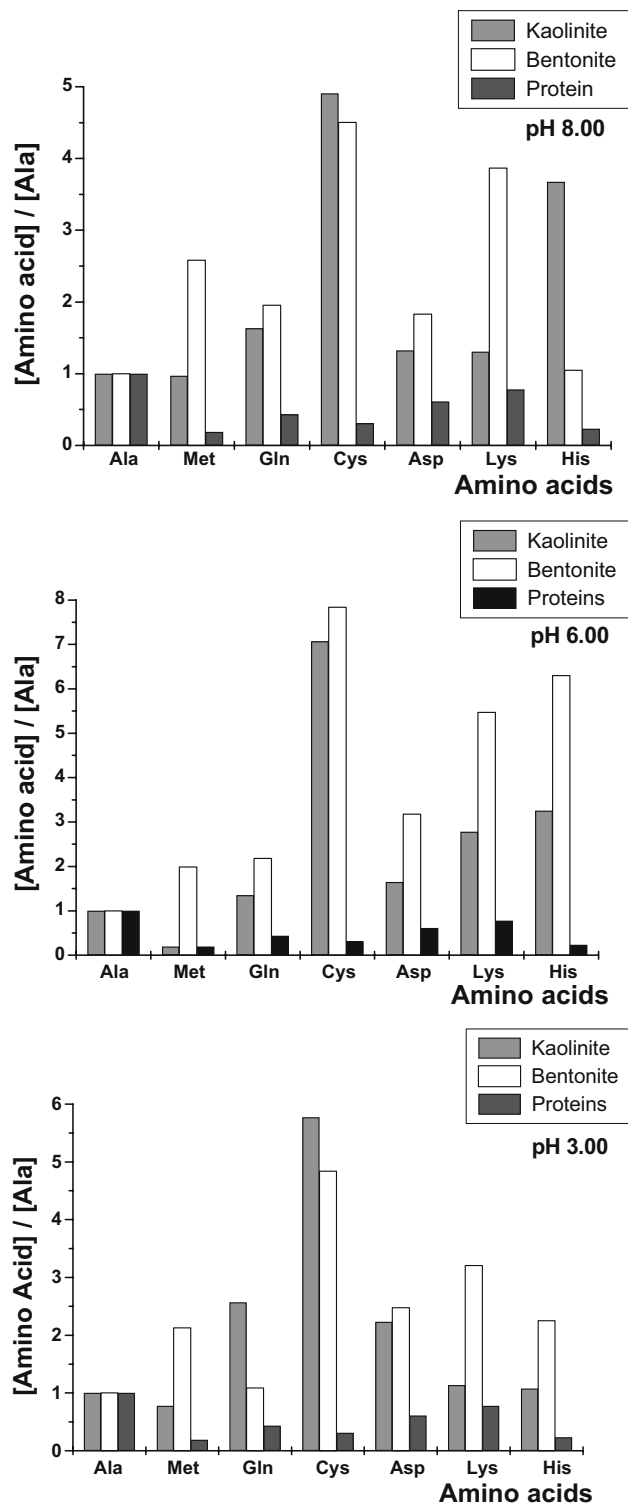
at a pH range of 3.00–6.00, and at pH 8.00 decreased to a level below that obtained at pH 3.00 (S.N.K. <0.05; Table 2). Rogacheva and Bobyrenko (1985) showed that adsorption of Lys on titanium dioxide varied inversely with pH. On the other hand, Ben-Taleb et al. (1994) noted that the maximum adsorption of Lys on hematite occurred close to its pI.

The amino acids that showed the greatest adsorption ($k > 1$) on kaolinite or bentonite were: Cys (both clays at all pH tested), Met (bentonite at pH 8.00), Asp (bentonite at pH 3.00), His (kaolinite at pH 8.00; bentonite at pH 6.00) and Lys (bentonite at all pH tested; Tables 1 and 2). In general, we can say that the amino acids with charged R groups (Asp, Lys, His) and Cys (polar, uncharged R group) were more adsorbed on kaolinite or bentonite than other amino acids (Ala, Met, Gln,) (Tables 1 and 2; Fig. 1). It should be pointed out that the adsorption of Cys was studied only on bare and octadecyl silica (Basiuk and Gromovoy 1996; Basiuk 2002) and silica gel/poly A (Mellersh and Wilkinson 2000). A review published by Lahav and Chang (1976) and various other papers mentioned in the introduction show the results of adsorption for several amino acids on clays/minerals/sediments. In almost all cases, amino acids with negatively or positively charged R groups are adsorbed more than other amino acids. Our experiments conducted under conditions close to those occurring naturally (sea water, wide pH range), show that amino acids with charged R groups and Cys are adsorbed more than other amino acids (Tables 1 and 2; Fig. 1). However, a study of the average occurrence of amino acids in proteins showed that 74% of the amino acids in today's proteins are amino acids with uncharged R groups (Cys, 2.8%; Klapper 1977). Thus, we propose that the adsorption of amino acids on minerals was not important for the prebiotic formation of peptides/proteins. If this notion is correct, we can then consider that one or more of the mechanisms discussed in the Introduction were important in the production of peptides/proteins with more amino acids with uncharged R groups than amino acids with charged R groups. In our opinion, the stability study of amino acids under hydrothermal conditions should be investigated more carefully. Andersson and Holm (2000) demonstrated that under buffered and non-buffered hydrothermal conditions (200°C, 50 bar) Ala was the most stable of four amino acids studied, and they also observed that Ala concentration increased in the early stage of experiments. Thus, this latter study could explain why Ala is the most abundant amino acid in proteins (Klapper 1977). It should also be pointed out that hydrothermal environments are useful in the synthesis of peptides as well as other biopolymers (Zamaraev et al. 1997; Imai et al. 1999; Mccollom et al. 1999; Ogasawara et al. 2000; Ogata et al. 2000; Washington 2000; Rushdi and Simoneit 2001; Alargov et al. 2002; Tsukahara et al. 2002; Yokoyama et al. 2003).

A systematic study using FT-IR spectroscopy was undertaken to better understand the interaction between clays and amino acids. For all amino acids (Ala, Met, Gln, Cys, Asp, Lys, His) adsorbed on clays (bentonite, kaolinite), FT-IR spectra did not change with variation in pH (range 3.00–8.00; data not shown). The FT-IR spectra of kaolinite, bentonite, solid amino acids and amino acids adsorbed on kaolinite and bentonite were recorded, and it was observed that the amino acids Ala, Lys, His and Met adsorbed on bentonite and kaolinite through the $-NH_3^+$ group (data not shown). This interaction was strong on bentonite and weak on kaolinite (data not shown). For Gln, there was a change in NH_2 to $-NH_3^+$ by the interaction of an electron pair of the NH_2 group with the surface of the clay (data not shown). FT-IR of Asp adsorbed on kaolinite and bentonite did not produce good spectra because of the low signal/noise ratio (data not shown).

Figure 2 shows the FT-IR spectra of kaolinite, bentonite, solid Cys and Cys adsorbed on kaolinite and bentonite. The FT-IR spectrum of solid Cys (Fig. 2a-b, b-b) shows bands at 646/838, 679, 776/1,429, 869, 1,347, 1,398, 1,521, 1,572, 1,623, 1,644 and 1,741 cm^{-1} which could be attributed to deformation of the COO^- group, stretching of C-S,

Fig. 1 Relative concentration of amino acids. The amount of Ala adsorbed on kaolinite or bentonite or the occurrence in the proteins (Klapper 1977) was taken as 1.00



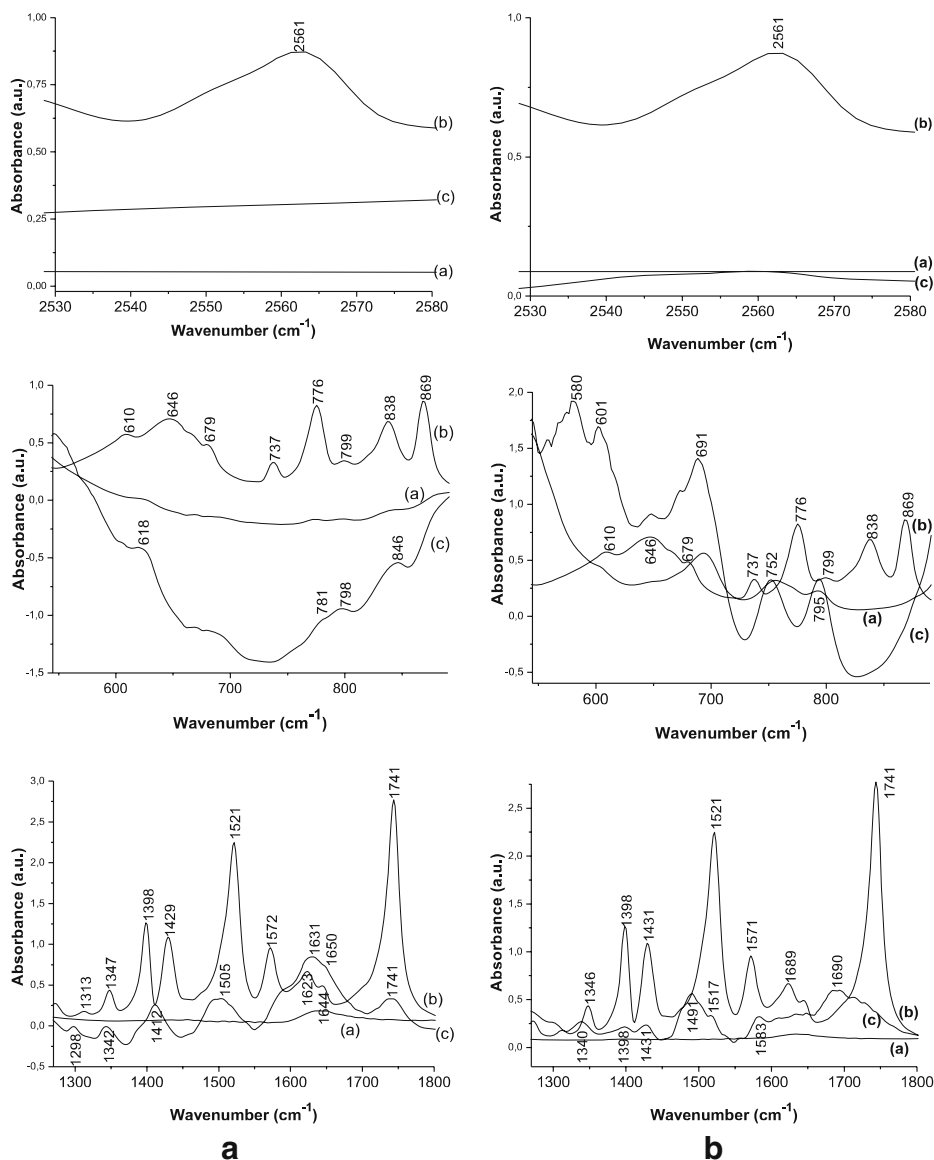


Fig. 2 FT-IR spectra of: **a** bentonite (a), solid cysteine (b) and cysteine adsorbed on bentonite (c); and **b** kaolinite (a), solid cysteine (b) and cysteine adsorbed on kaolinite (c). Clays samples were tumbled for 24 h with seawater and saturated cysteine solution dissolved in seawater. All samples were spun for 15 min at 2,000 rpm, and the solids were dried in an oven at 40°C for 24 h

deformation of the CH_2 group, stretching of C-C, symmetric deformation of the NH_3 group, symmetric stretching of COO^- , deformation of the N-H group, asymmetric deformation of $-\text{NH}_3^+$, asymmetric deformation of $-\text{NH}_3^+$, asymmetric stretching of COO^- and stretching of C=O, respectively (Stewart and Fredericks 1999; Wolpert and Hellwig 2006). The weak band near $2,561\text{ cm}^{-1}$ could be attributed to the SH group in the Cys molecule (Pawlukojé et al. 2005; Aryal et al. 2006; Wolpert and Hellwig 2006). In the region of 3,000–

$3,600\text{ cm}^{-1}$, the spectra were not well defined because of the low signal/noise ratio. The spectrum of Cys adsorbed on bentonite showed that the bands at $1,429$ and $1,572\text{ cm}^{-1}$ vanished, those at $1,347$ and $1,741\text{ cm}^{-1}$ decreased in intensity, those at 838 , $1,398$, $1,623$ and $1,644\text{ cm}^{-1}$ shifted to 846 , $1,412$, $1,631$ and $1,650\text{ cm}^{-1}$, and that at $1,521\text{ cm}^{-1}$ decreased in intensity and shifted to $1,505\text{ cm}^{-1}$. The bands due to stretching of S-H and C-S were not observed in the spectrum of Cys adsorbed on bentonite, because of the strong sulfur/bentonite interaction (Fig. 2a-c). For the samples of Cys adsorbed on kaolinite, the spectra show that the band at $2,561\text{ cm}^{-1}$ decreased in intensity, and that the band at 679 cm^{-1} was not possible to observe due to the strong band at 691 cm^{-1} of kaolinite (Fig. 2b-c). It is likely that the sulfur/kaolinite interaction is as strong as that of sulfur-bentonite. The following were also observed: the bands at 776 , 838 , 869 , $1,431$ and $1,571\text{ cm}^{-1}$ vanished; the bands at $1,346$, $1,398$ and $1,741\text{ cm}^{-1}$ decreased in intensity; and the band at $1,521\text{ cm}^{-1}$ decreased in intensity and split into two bands at $1,491$ and $1,517\text{ cm}^{-1}$ (Fig. 2b-c). Thus, the Cys/clay interaction occurs through the sulfhydryl and amine groups, demonstrating its complexity.

The mechanism for the formation of peptide bonds on clays using dry/wet cycles has been proposed where the amino acids interact with clay through the carbonyl group (White et al. 1984; Bujdák and Rode 2001). However, the FT-IR spectra of the amino acids adsorbed on clays showed that the amine group was the group involved in the adsorption of amino acids.

The spectrum of Cys adsorbed on bentonite showed major variations among the amino acids studied here. Thus, a study using X-ray diffractometry was undertaken to better understand this adsorption. The X-ray diffractometry data show that pH affects the bentonite interlayer. Interlayer expansion occurring at pH 3.00, 6.00 and 8.00 was 14.1 , 14.9 and 13.9 Å , respectively (data not shown). For samples of Cys adsorbed on bentonite, expansion of the interlayer was greater due to the inclusion of Cys. The expansion of the interlayer was greater at pH 3.00 (18.3 Å) and 8.00 (17.3 Å), and no expansion (15.2 Å) was observed at pH 6.00. Interlayer expansion at pH 3.00 of Cys/bentonite was greater than that of samples of ethylene glycol/bentonite saturated with Mg (17.7 Å). These results demonstrate the higher affinity of the bentonite interlayer for Cys (Borchardt 1989; Moore and Reynolds 1989).

Fig. 3 RT Mössbauer spectrum for bentonite

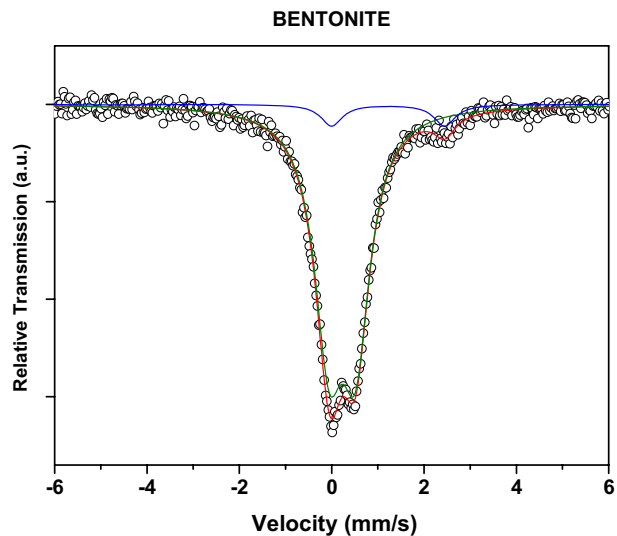
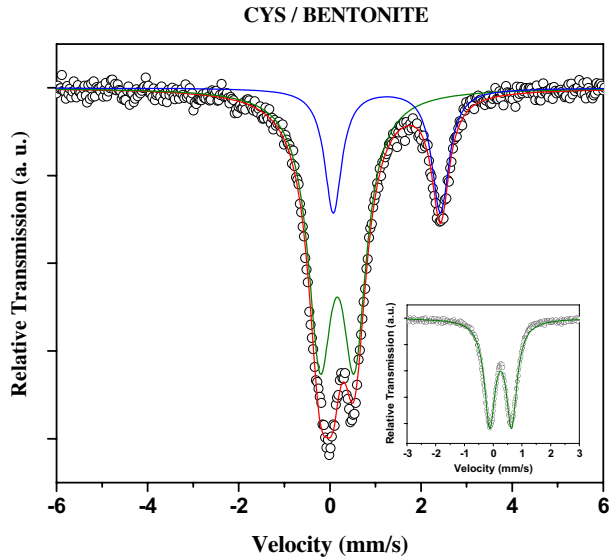


Fig. 4 RT Mössbauer spectrum for the Cys adsorbed on bentonite. The insert shows the Cys/ Fe^{3+} spectrum



The results of X-ray diffractometry showed that Cys was included in the bentonite interlayer and chemical analysis of bentonite showed 2.7% as Fe_2O_3 , and as pointed out by several authors (Hartman 1975; Wächtershäuser 1988; Kaschke et al. 1994), the interaction of sulfur compounds/iron played an important role in prebiotic chemistry. Thus, a study using Mössbauer spectroscopy was undertaken to better understand the possible iron/Cys interaction. Figure 3 shows the RT Mössbauer spectrum for bentonite. The spectrum was fitted with two doublets, the major component corresponding to the ferric ion and the minor one belonging to the ferrous ion. This is consistent with earlier reported results where both valences of iron, Fe^{3+} and Fe^{2+} , were detected (Oliveira et al. 2003). The Mössbauer spectrum for the sample with adsorbed Cys is shown in Fig. 4. Again, two doublets can be observed comprising the full spectrum, although with a large increase (~20%) due to the ferrous contribution. This means that Cys was able to partially reduce iron present in bentonite. This result is similar to that which occurs with aconitase where the ferric ions are reduced to Fe 2.5, this means that one electron is shared by two ferric ions (Cowan 1993).

Table 3 Mössbauer hyperfine parameters and subspectral areas for bentonite samples, without and with cysteine adsorbed

Sample	Temperature (K)	Subspectrum	IS ^a (mm/s ; ± 0.02)	QS (mm/s; ± 0.02)	Γ (mm/s; ± 0.2)	Area (%; ± 0.3)
Without Cys	300	Doublet (Fe^{3+})	0.23	0.58		93.3
		Doublet (Fe^{2+})	1.22	2.45		6.7
With Cys	300	Doublet (Fe^{3+})	0.27	0.75	0.64	73.0
		Doublet (Fe^{2+})	1.36	2.35	0.45	27.0

^a Relative to α -Fe foil at room temperature

The insert shows the Cys/Fe³⁺ RT spectrum. The hyperfine parameters and subspectral areas obtained from these fits are presented in Table 3.

Conclusion

The amount of Ala, Cys and Asp adsorbed on kaolinite as well as Cys and Asp adsorbed on bentonite did not change over the pH range studied here.

In general, the amino acids with charged R groups (Asp, Lys, His) and Cys (polar, uncharged R group) were more adsorbed on kaolinite or bentonite than other amino acids (Ala, Met, Gln). These results raise some questions about the role of minerals in providing a concentration mechanism for amino acids: What were the mechanisms involved in producing peptides/proteins with more amino acids with uncharged R groups than charged R groups and Cys? Should we expect the composition of amino acids adsorbed on minerals to reflect that of present day proteins? Was the adsorption of amino acids on minerals important for the origin of life?

The FT-IR spectra showed that the adsorption of Ala, Met, Gln, Asp, Lys and His on the clays (bentonite, kaolinite) occur through the amine group. However, the Cys/clay interaction occurs through the sulfhydryl and amine groups, demonstrating the complexity of the Cys/clay interaction.

The X-ray diffractometry data showed that pH affected the bentonite interlayer and that the expansion at pH 3.00 of Cys/bentonite was greater than the expansion of ethylene glycol/bentonite saturated with Mg. These results showed the higher affinity of bentonite interlayer for Cys.

The Mössbauer spectrum for the sample with absorbed Cys showed a large increase (~20%) in ferrous ions. This means that Cys was able to partially reduce the iron present in bentonite. This result is similar to that which occurs with aconitase where the ferric ions are reduced to Fe 2.5. This result shows that Cys could have played an important role in prebiotic chemistry besides just being one of the amino acids in the peptides/proteins.

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References

- Alargov DK, Deguchi S, Tsujii K, Horikoshi K (2002) Reaction behaviors of glycine under super and subcritical water conditions. *Orig Life Evol Biosph* 32:1–12
- Andersson E, Holm NG (2000) The stability of some selected amino acids under attempted redox constrained hydrothermal conditions. *Orig Life Evol Biosph* 30:9–23
- Appel C, Ma LQ, Rhue RD, Kennelley E (2003) Point of zero charge determination in soils and minerals via traditional methods and detection of electroacoustic mobility. *Geoderma* 113:77–93
- Aryal S, Remant BKC, Dharmaraj N, Bhattari N, Kim CH, Kim HY (2006) Spectroscopic identification of S–Au interaction in cysteine capped gold nanoparticles. *Spectrochim Acta Part A* 63:160–163
- Aufdenkampe AK, Hedges JI, Richey JE, Krusche AV, Uerena AV (2001) Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. *Limnol Oceanogr* 46: 1921–1935
- Basiuk VA (2002) Adsorption of biomolecules on silica. In: Somassundaran P (ed) *Encyclopedia of surface and colloid science*. Marcell Dekker, New York, p 277

- Basiuk VA, Gromovoy TY (1996) Comparative study of amino acid adsorption on bare and octadecyl silica from water using high-performance liquid chromatography. *Colloids Surf A Physicochem Eng Asp* 118: 127–140
- Bebić J, Schoonen MAA (2000) Pyrite surface interaction with selected organic aqueous species under anoxic conditions. *Geochem Trans* 1:47–53
- Ben-Taleb A, Vera P, Delgado AV (1994) Electrokinetic studies of monodisperse hematite particles: effects of inorganic electrolytes and amino acids. *Mater Chem Phys* 37:68–75
- Bernal JD (1951) *The physical basis of life*. Routledge and Kegan Paul, London
- Blocher M, Liu D, Walde P, Luisi PL (1999) Liposome assisted selective polycondensation of α -amino acids and peptides. *Macromolecules* 32:7332–7334
- Blocher M, Liu D, Walde P, Luisi PL (2000) Liposome assisted selective polycondensation of α -amino acids and peptides: the case of charged liposomes. *Macromolecules* 33:5787–5796
- Böhler C, Hill AR Jr, Orgel LE (1996) Catalysis of the oligomerization of *o*-phospho-serine, aspartic or glutamic acid by cationic micelles. *Orig Life Evol Biosph* 26:1–5
- Borchardt G (1989) Smectites. In: Dixon JB, Weed SB (eds) *Minerals in soil environments*, 2 edn. Soil Science Society of America, Madison, pp 675–727
- Bujdák J, Rode BM (2001) Activated alumina as an energy source for peptide bond formation: consequences for mineral mediated prebiotic processes. *Amino Acids* 21:281–291
- Churchill H, Teng H, Hazen RM (2004) Correlation of pH dependent surface interaction forces to amino acid adsorption: implications for the origin of life. *Am Mineral* 89:1048–1055
- Cowan JA (1993) *Inorganic Biochemistry an Introduction*. VCH, New York, pp 154–155
- Darnell J, Lodish H, Baltimore D (1990) *Molecular cell biology*. Scientific American Books, New York, p 48
- De Santana H, Toni LRM, Benetoli LOB, Zaia CTBV, Rosa M Jr, Zaia DAM (2006) Effect in glyphosate adsorption on clays and soils heated and characterization by FT-IR Spectroscopy. *Geoderma* 136:738–750
- Ding X, Henrichs SM (2002) Adsorption and desorption of proteins and polyamino acids by clay minerals and marine sediments. *Mar Chem* 77:225–237
- Fisher LJ, Buting SL, Rosenberg LE (1963) A modified ninhydrin colorimetric method for determination of plasma alpha-amino nitrogen. *Clin Chem* 9:573–581
- Franchi M, Ferris JP, Gallori E (2003) Cations as mediators of the nucleic acids on clay surfaces in prebiotic environments. *Orig Life Evol Biosph* 33:1–16
- Grzegorzczak DS, Carta G (1996) Adsorption of amino acids on porous polymeric adsorbents 1 Equilibrium. *Chem Eng Sci* 51:807–817
- Imai EI, Honda H, Hatori K, Matsuno K (1999) Autocatalytic synthesis of oligoglycine in a simulated submarine hydrothermal system. *Orig Life Evol Biosph* 29:249–259
- Hartman H (1975) Speculation on the origin and evolution of metabolism. *J Mol Evol* 4:359–370
- Henrichs SM, Sugai SF (1993) Adsorption of amino acids and glucose by sediments of Resurrection Bay, Alaska, USA-Functional group effects. *Geochim Cosmochim Acta* 57:823–835
- Holm NG, Andersson E (2005) Hydrothermal simulation experiments as a tool for studies of the origin of life on earth and other terrestrial planets: a review. *Astrobiology* 5:444–460
- Holm NG, Dowler MJ, Wadsten T, Arrhenius G (1983) β -FeOOH.Cl_n (akaganéite) and Fe_{1-x}O (wüstite) in hot brine from the Atlantis II Deep (Red Sea) and uptake of amino acids by syntentic β -FeOOH.Cl_n. *Geochim Cosmochim Acta* 47:1465–1470
- Holm NG, Dumont M, Ivarsson M, Kohn C (2006) Alkaline fluid circulation in ultramafic rocks and formation of nucleotide constituents: a hypothesis. *Geochem Trans* 7:7
- Kalra S, Pant CK, Pathak HD, Mehta MS (2000) Adsorption of glycine and alanine on montmorillonite with or without coordinated divalent cations. *Indian J Biochem Biophys* 37:341–346
- Kaschke M, Russell MJ, Cole WJ (1994) [FeS/FeS₂] A redox system for the origin of life. *Orig Life Evol Biosph* 24:43–56
- Klapper MH (1977) Independent distribution of amino acids near neighbor pairs into polypeptides. *Biochem Biophys Res Commun* 78:1018–1024
- Lahav N, Chang S (1976) The possible role of solid surface area in condensation reactions during chemical evolution: reevaluation. *J Mol Evol* 8:357–380
- Lehninger AL, Nelson DL, Cox MM (1993) *Principles of Biochemistry*. Worth, New York, NY, p 113
- Luisi PL, Walde P, Blocher M, Liu D (2000) Research on the origin of life: membrane assisted polycondensation of amino acids and peptides. *Chimia* 54:52–53
- Mccollom TM, Ritter G, Simoneit BRT (1999) Lipid synthesis under hydrothermal conditions by Fischer-Tropsch type reactions. *Orig Life Evol the Biosph* 29:153–166
- Mellersh A, Wilkinson AS (2000) RNA bound to a solid phase can select an amino acid and facilitate subsequent amide bond formation. *Orig Life Evol the Biosph* 30:3–7
- Meng M, Stievano L, Lambert JF (2004) Adsorption and thermal condensation mechanisms of amino acids on oxide supports. 1. Glycine on silica. *Langmuir* 20:914–923

- Montluçon DB, Lee C (2001) Factors affecting lysine sorption in a coastal sediment. *Org Geochem* 32:933–942
- Moore DM, Reynolds RC Jr (1989) X-ray diffraction and the identification analysis of clays minerals, 2 edn. Oxford University Press, New York, p 378
- Ogasawara H, Yoshida A, Imai EI, Honda H, Hatori K, Matsuno K (2000) Synthesizing oligomers from monomeric nucleotides in simulated hydrothermal environments. *Orig Life Evol Biosph* 30:519–526
- Ogata Y, Imai EI, Honda H, Hatori K, Matsuno K (2000) Hydrothermal circulation of seawater through hot vents and contribution of interface chemistry to prebiotic synthesis. *Orig Life Evol Biosph* 30:527–537
- Oliveira LCA, Rios RVRA, Fabris JD, Sapag K, Garg VK, Lago RM (2003) Clay-iron oxide magnetic composites for the adsorption of contaminants in water. *Appl Clay Sci* 22:169–177
- Paecht-Horowitz M (1978) Clay catalyzed polymerization of amino acid adenylates and its relationship to biochemical reactions. *Origin of Life* 289–295
- Parks GA (1967) Aqueous surface chemistry of oxides and complex oxide minerals. Iso-electric point and zero point of charge In: Stumm W (ed) *Equilibrium concepts in natural water systems*. Advanced in Chemistry series no 67. American Chemical Society, Washington DC, pp 121–160
- Pawlukojé A, Leciejewicz J, Ramírez-Cuesta AJ, Nowicka-Scheibe J (2005) L-cysteine: neutron spectroscopy, Raman, IR and ab initio study. *Spectrochim Acta Part A* 61:2474–2481
- Plankensteiner K, Righi A, Rode BM (2002) Glycine and diglycine as possible catalytic factors in the prebiotic evolution of peptides. *Orig Life Evol Biosph* 32:225–236
- Rogacheva NV, Bobyrenko Yu Ya (1985) Adsorption of some amino acids on titanium dioxide surface. *Russ J Phy Chem* 59:769–770
- Rushdi A, Simoneit BRt (2001) Lipid formation by aqueous Fischer-Tropsch type synthesis over a temperature range of 100 to 400°C. *Orig Life Evol Biosph* 31:103–118
- Sowerby SJ, Petersen GB, Holm NG (2002) Primordial coding of amino acids by adsorbed purine bases. *Orig Life Evol Biosph* 32:35–46
- Stewart S, Fredericks PM (1999) Surface enhanced Raman spectroscopy of amino acids on an electrochemically prepared silver surface. *Spectrochim Acta Part A* 55:1641–1660
- Suwanachot Y, Rode BM (1999) Mutual amino acid catalysis in salt induced peptide formation support this mechanism's role in prebiotic peptide evolution. *Orig Life Evol Biosph* 29:463–471
- Tanaka H, Miyajima K, Nakagaki M, Shimabay S (1989) Interaction of aspartic acid, alanine and lysine with hydroxyapatite. *Chem Pharm Bull* 37:2897–2901
- Tessis AC, Penteado-Fava A, Pontes-Buarque M, de Amorin HS, Bonapace JAP, de Souza-Barros F, Vieyra A (1999) Pyrite suspended in artificial sea water catalyzes hydrolysis of adsorbed ATP: enhancing effect of acetate. *Orig Life Evol Biosph* 29:361–374
- Tsukahara H, Imai EI, Honda H, Hatori K, Matsuno K (2002) Prebiotic oligomerization on or inside lipid vesicles in hydrothermal environments. *Orig Life Evol Biosph* 32:13–21
- Wächtershäuser G (1988) Before enzymes and templates: theory of surface metabolism. *Microbiol Rev* 52:452–484
- Washington J (2000) The possible role of volcanic aquifers in prebiologic genesis of organic compounds and RNA. *Orig Life Evol Biosph* 30:53–79
- White DH, Kennedy RM, Macklin J (1984) Acyl silicates aluminates as activated intermediates in peptide formation on clays. *Orig Life* 14:273–278
- Wolpert M, Hellwig P (2006) Infrared spectra and molar absorption coefficients of the 20 alpha amino acids in the spectral range from 1,800 to 500 cm⁻¹. *Spectrochim Acta Part A* 64:987–1001
- Yanagawa H, Kojima K, Ito M, Handa N (1990) Synthesis of polypeptides by microwave heating. I. Formation of polypeptides during repeated hydration–dehydration cycles and their characterization. *J Mol Evol* 31:180–186
- Yokoyama S, Koyama A, Nemoto A, Honda H, Imai EI, Hatori K, Matsuno K (2003) Amplification of diverse catalytic properties of evolving molecules in a simulated hydrothermal environment. *Orig Life Evol Biosph* 33:589–595
- Zaia DAM (2004) A review of adsorption of amino acids on minerals: was it important for origin of life? *Amino Acids* 27:113–118
- Zaia DAM, Zaia CTBV (2006) Adsorption of amino acids on minerals and the origin of life. *Quim Nova* 29:786–789
- Zaia DAM, Ribas KCL, Zaia CTBV (1999) Spectrophotometric determination of cysteine and/or carbocysteine in a mixture of amino acids, shampoo, and pharmaceutical products using *p*-benzoquinone. *Talanta* 55:1003–1010
- Zaia DAM, Vieira HJ, Zaia CTBV (2002) Adsorption of L-amino acids on sea sand. *J Braz Chem Soc* 13:679–681
- Zamaraev KI, Romannikov VN, Salganik RI, Wlassoff WA, Khramtsov VV (1997) Modeling of the prebiotic synthesis of oligopeptides: silicate catalysts help to overcome the critical stage. *Orig Life Evol Biosph* 27:325–337