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Surface Force Analysis of Pyrite (FeS_2): Its Reactivity to Amino Acid Adsorption

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Abstract

It is well known that mineral surfaces play an important role as catalysts for abiotic polymerization of amino acids to form peptides, which are the main components of the first self-replicating system. Understanding the mechanism behind the adsorption of simple amino acids on mineral surfaces is a topic of great interest not only in field of prebiotic evolution and but also in many other branches of material sciences. Various clay minerals have been suggested for studying how organic molecules were first synthesized in a prebiotic "inorganic" environment. Among them, pyrite (FeS_2) is one of the most potential minerals as it possesses a highly reactive surface to drive molecular adsorption in prebiotic chemistry reactions. Recent theoretical experiments suggest that amino acids are adsorbed on the pyrite surface depending on its surface structures. However, these results have not been tested experimentally, and the exact mechanism of the specific interactions on this mineral has not been fully resolved yet at the molecular level. In this work, through quantitative force analysis with atomic force microscope (AFM) in which a single amino acid residue was mounted on the tip apex of AFM probe, we were able to find the reaction sites and study the interaction forces between the amino acid and the pyrite surface. Our results of Raman spectroscopic studies and force measurements with a well-designed AFM probe demonstrated for the first time that pyrite provided higher adsorption probabilities of amino acid residues for the chemical reactions at surfaces.

Keywords

Pyrite, Mineral-Organic Interface, Atomic Force Microscopy, Amino Acid Adsorption

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1. Introduction

Understanding the interaction between biomolecules, such as amino acids, peptides, or proteins, and mineral surfaces is currently a topic of importance in the fields of surface chemistry, catalysis, and prebiotic chemistry. Minerals are well established as catalysts for abiotic polymerization and can be very promising surfaces for studying biomolecule-surface processes [1]-[3]. Among such minerals, pyrite (FeS_2) is one of the most important and abundant sulfide minerals on earth. A theory called "iron-sulfur world" [4] [5] by Wächtershäuser proposed that the first reactions that led to the formation of amino acids did not occur in a bulk solution in the oceans (prebiotic soup theory) but on the surface of pyrite because such surfaces have the potential to facilitate prebiotic polymerization. Moreover, recent molecular dynamics studies [6]-[8] have suggested that pyrite surfaces resulting from crystallographic structure changes could selectively bind to simple amino acid residues. However, these theoretical results regarding simple amino acid adsorption on pyrite have not been proved experimentally. To elucidate the adsorption mechanisms of simple prebiotic molecules on pyrite surfaces, it is essential to investigate the adsorption behavior at such interfaces on the molecular scale. In addition, understanding the adsorption of simple amino acids on pyrite surfaces would enhance our knowledge of the means through which peptides bond to form proteins. Therefore, we aim to investigate the affinity of simple amino acid adsorption on pyrite and to experimentally reveal the effects of pyrite surfaces on molecular bonding.

In this work, we used Raman spectroscopy to identify the surface structures of pyrite and a commercial AFM system equipped with a liquid cell (MFP-3D, Asylum Research, Santa Barbara, CA) to study the interaction forces between amino acids and pyrite surfaces at the molecular level. AFM is a powerful tool to perform highly precise force measurements, and it allows detailed study of molecular interactions, which is not possible with other techniques [9]-[14]. We chose to study lysine and other simple amino acids that are considered to have been present on primordial earth [10]-[12]. A well-established tip modification method was used to immobilize the amino acid molecules on AFM tips in preparation for the force measurements.

2. Materials and Methods

Tip Modification

Commercial soft cantilevers (OMCL-TR400 PB-1, both sides gold-coated) with nominal spring constants of 0.02 N/m were used for the tip modification. In the AFM probe functionalisation methods (**Figure 1**) [14] [15], the cantilevers were first introduced by mixing two types of thiol reagents in ethanol: 1,8-octanedithiol (2 mM) and 6-mercapto hexan-1-ol (20 mM) for more than 18 h. The mixed solutions were used to increase the probability of single molecular events occurring by controlling the densities of molecules on the tips as well as to prevent molecular aggregation on the tip surfaces. After washing with ethanol, 1 mg/ml of the cross-linker molecules in toluene N-hydroxysuccinimide ester-polyethylene glycol-maleimide (MAL-PEG_n-NHS, n = 24, Mw = 1340, purchased from Quanta BioDesign, Ltd., Boston, MA) were anchored to the tips for 60 min by reacting their maleimide ends with -SH of alkanethiol to form stable covalent bonds. They were then washed several times with phosphate buffered solution (PBS) to remove the unreacted cross-linkers. After that, the amino acid residues were cross-linked to the tips through the free NHS ends of the PEG cross-linker molecules by immersing the tips into 1 mM of amino acid solution in PBS (pH = 7.4) for 2 h at room temperature. Finally, the modified tips were again washed with PBS to remove the unbound molecules for the force measurements.

In the case of immobilizing lysine, it contains "epsilon" and "alpha" amino groups. Basically, NHS-reactive groups of PEG cross linkers should react with both amino groups. However, we assume that the epsilon amino group has a higher pKa value (pKa = 10.5) than that of the alpha amino group (pKa = 9), which often participates in hydrogen bonding, and in a buffer solution that does dissociate the epsilon amino, but not the alpha amine. Therefore, alpha amine should react with the NHS groups of PEG cross linker.

3. Results

3.1. Sample Preparation

A single crystalline cube (100) of commercially available natural pyrite (Navajun, Spain) (**Figure 2(a)**) was used in our experiments. This mineral was cut into small slices (1 cm × 1 cm) (**Figure 2(b)**) using a Lab Cutter device (MC-120, Maruto) for the Raman and AFM experiments. After cutting, the substrates were cleaned by

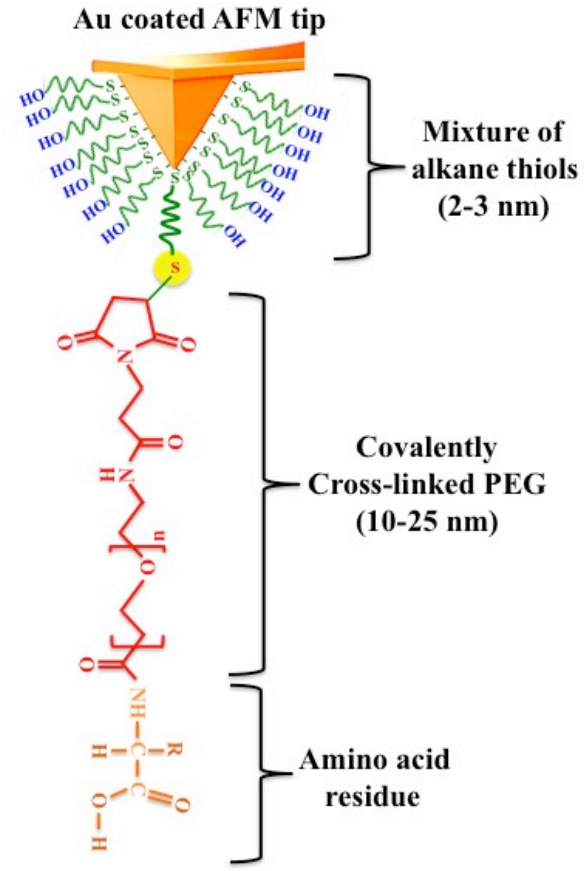
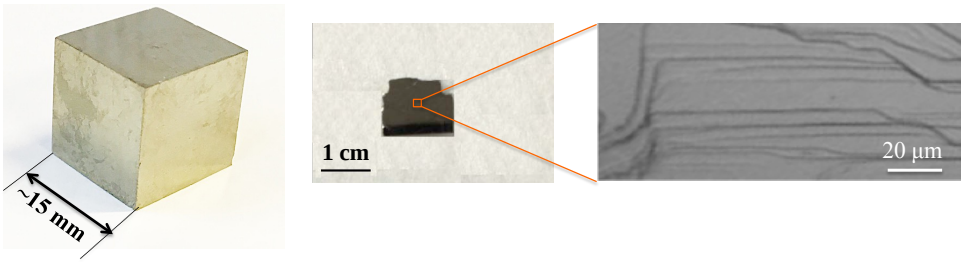


Figure 1. Schematic AFM tip modification by amino acid residues. Gold-coated AFM tip with nominal radius of ~20 nm was chemically modified by amino acid molecules through MAL-PEG$_n$-NHS cross-linker. The maleimide group reacts with sulfhydryls to form stable thioether bonds. The N-hydroxysuccinimide ester (NHS) group reacts specifically with N-terminal amino groups at pH = 7 - 9 to form stable amide bonds.



(a) (b) (c)

Figure 2. (a) Image of natural pyrite mineral (Navajun, Spain); (b) Image of pyrite substrate after cutting; (c) Optical microscope image of the outermost surface of the pyrite showing the step-like structure of single crystal planes.

ultrasonication in ethanol solution and deionized water to remove the contaminants from the surface. An optical microscope shows the terrace-like structure of the outermost surface of pyrite used in our experiments (**Figure 2(c)**).

3.2. Raman Spectral Characterization of Natural Pyrite Surface

We first performed the Raman spectroscopic analysis of a single crystalline cube of natural pyrite to identify the different crystallographic structure sites on the surface. After that, force measurements were conducted on pyrite

surface. An optically reflected image of the pyrite surface is shown in **Figure 3(a)**, revealing step edges, terraces, and kinks. Raman spectra were measured at position A in **Figure 3(a)** (diameter of 1.4 μm) using a 532 nm laser line for excitation. The experiment revealed two dominant peaks at approximately 345 cm^{-1} and 380 cm^{-1}, which are attributable to the vibrational (E_g) and stretching (A_g) modes, respectively, of sulfur dumbbells [16]. A small peak at ~435 cm^{-1} corresponds to a combination of the stretching and vibrational modes (T_g) (**Figure 3(b)**).

Raman measurements were conducted at a different position indicated by B in **Figure 4(a)** to check the reliability of the results. We observed the same spectra with three modes as measured for position A. However, there are considerable differences between the two spectra from positions A and B, particularly in terms of the frequencies, intensities, and widths of their A_g mode peaks (**Figure 4**).

At position B, the frequency of A_g mode peak is lower and the width is broadened compared to the corresponding peak at position A. The intensity A_g mode peak decreased significantly at position B. These differences originate from the increased bond length between the sulfur dumbbells. The increased bond length might be particularly attributable to the desorption of sulfur atoms from the surface, indicating the presence of non-stoichiometric pyrite (FeS_{2-x}) [17]. In this case, we performed thermal desorption spectroscopy (TDS) to check the desorption of sulfur atoms from pyrite surface, and the results showed the preferential desorption of sulfur atoms from the pyrite surface (Supplementary Information, **Figure S1**) indicating that sulfur vacancy sites are intrinsic in pyrite.

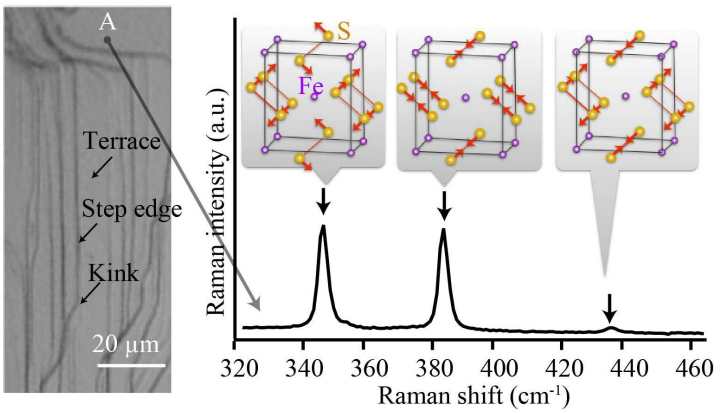


Figure 3. (a) Optical microscope image of outer surface of pyrite. Step edge, terrace, and kink on the sample surface are marked by arrows; (b) Raman spectra of pyrite outer surface indicated by point A in the optical image.

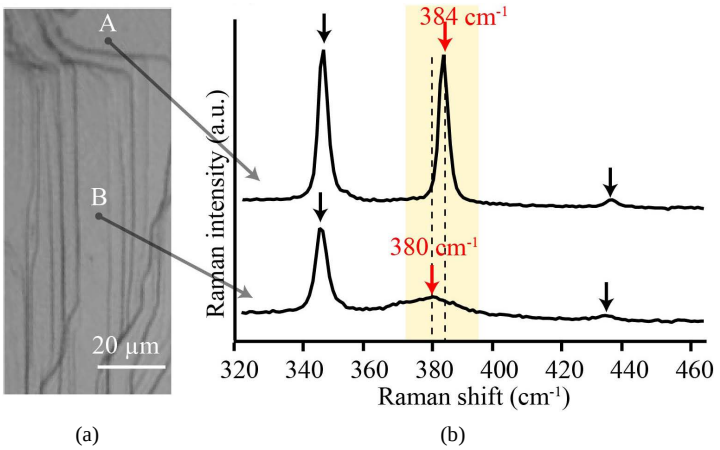


Figure 4. (a) Optical microscope image of outer surface of pyrite; (b) Raman spectra of pyrite outer surface measured at positions A and B. Considerable differences between the two spectra, particularly in terms of the frequencies, intensities, and widths of their A_g mode peaks, are observed.

3.3. General Force Measurements

After identifying the presence of non-stoichiometric sites on pyrite by Raman measurements, we intend to study the adsorption behavior of amino acid by using AFM. All force measurements were conducted at room temperature in a PBS solution (pH = 7.4) to minimize the nonspecific interactions between the probe and the substrate. Several hundred force-distance (F-D) curves were recorded with at least two amino acid modified AFM tips by lowering each modified AFM tip onto the measured surface to a maximum load of 400 pN.

3.3.1. Force Measurements of Lysine on Pyrite

Specific adhesion events with moderate frequency were observed at a loading rate of 6 nN/s. Specific adhesion events can be identified by typical extension of the tethered cross-linker following detachment of the amino acid residue from the surface. The extension length (the length at the maximum physically possible extension of the cross linker polymer) ranges from approximately 15 to 25 nm (**Figure 5**). This range is reasonable given the total length of the molecules used to mount the amino acid molecules on the tips. Since the molecular length of the amino acid residue was too small, most of the extension must have resulted from the linker molecules, which could have been extended to a total length of approximately 12 - 15 nm. The cross-linker N-hydroxysuccinimide ester-polyethylene glycol-maleimide (NHS-PEG-MAL) has a standard extended length of ~10 nm and short thiol molecules are ~2 nm. However, the extension lengths vary since molecules can exist in different conformations, which is difficult to control.

For examining the reliability of the experimental results, several force curves within at least three different functionalized tips are displayed vertically in **Figure 6(a)**. Furthermore, the adhesion forces in these experiments recorded range from about 100 to 300 pN, and 10% of the specific adhesion events are accounted for from several hundred (~600) F-D curves. This low probability of adhesion provides evidence that single-molecule interactions with the substrate were indeed measured [18]. For the number of adhesion events recorded on

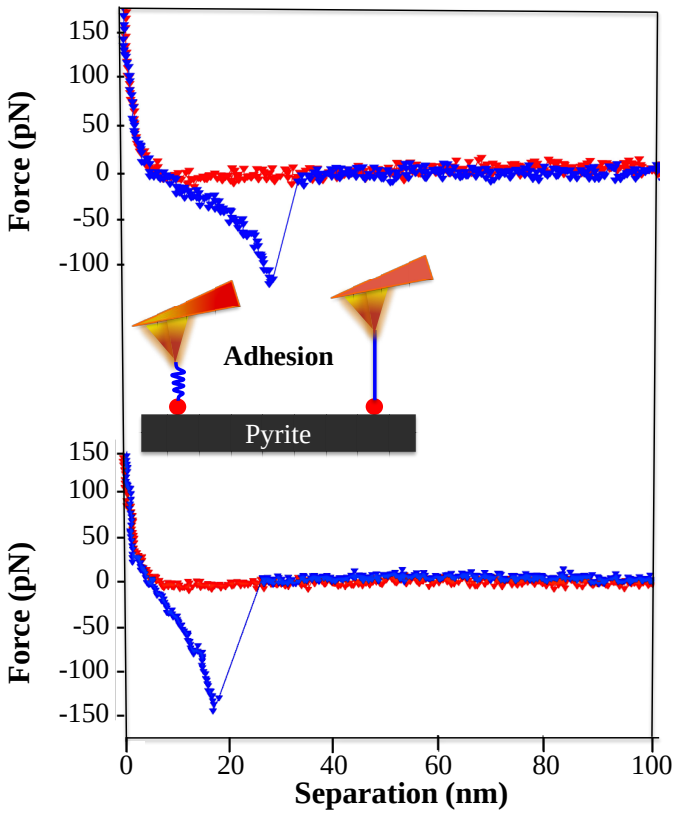
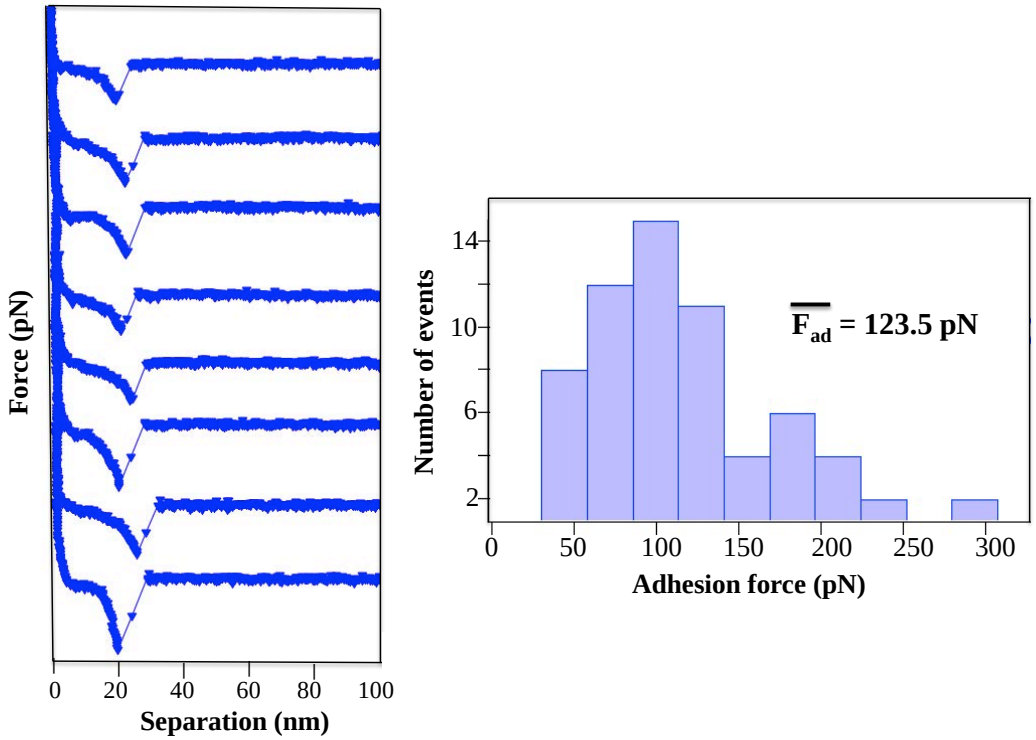


Figure 5. Force measurement between lysine and pyrite. Typical single-molecule F-D curves of AFM tip modified with lysine on pyrite surface. Specific adhesion events are evident during AFM probe retraction; Red and blue traces indicate signals upon approach and retraction from surface, respectively.



(a) (b)

Figure 6. (a) Typical F-D curves during retraction of an AFM tip modified with lysine on pyrite are displayed vertically. These force curves were produced from three different functionalized tips with the same loading rate of 6 nN/s; (b) Unbinding force histogram. MPF for lysine on pyrite surface at loading rate of 6 nN/s (pH = 7.4). Average adhesion force was calculated by using Gaussian fit and shows mean of 123.5 pN.

pyrite, we constructed a histogram by plotting the number of events versus the rupture force. A Gaussian fit applied to the histogram indicated the average adhesion force, referred to as the most probable force (MPF) (**Figure 6(b)**). Only those rupture events showing the extended length in reasonable range (10 - 30 nm) are included in the given histogram.

3.3.2. Force Measurements of Lysine on Mica

We further investigated the interactions between lysine and mica as a reference experiment and observed no interactions when the same modified tips that were used for the pyrite substrate were employed (**Figure 7**). Measurements were conducted in a PBS solution (pH 7.4) at loading rate of 6 nN/s under the same conditions. Therefore, the adhesion events measured in this experiment indeed originated from the interactions between the amino acid molecules and the pyrite surfaces, rather than from the interactions between the substrate and the unsuccessfully modified tips.

3.3.3. Force Measurements of Other Amino Acids on Pyrite

Additionally, we could observe the specific adsorption of other amino acids on the pyrite surfaces. We chose to study sulfur containing amino acids, cysteine and methionine (**Figure 8**). Measurements were conducted in a PBS solution (pH 7.4) at loading rate of 6 nN/s. No big difference among lysine, cysteine, and methionine was observed in the adhesion force range (120 - 200 pN). The results were consistent with our TDS measurements in which amino acids showed chemisorption peaks (**Figure 9**).

3.4. TDS Measurements

In TDS experiments, pyrite was immersed into 100 mM of amino acid solutions for one day. After that, the substrate was heated from 50˚C to 350˚C at 10˚C/min under less than 10^{-5} Pa. The first peak indicates weak physical

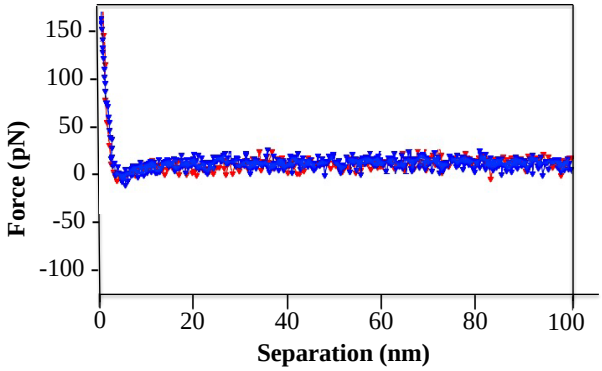


Figure 7. Typical F-D curves of AFM tips modified with lysine on mica surface. Red and blue traces indicate signals upon approach and retraction from surface, respectively.

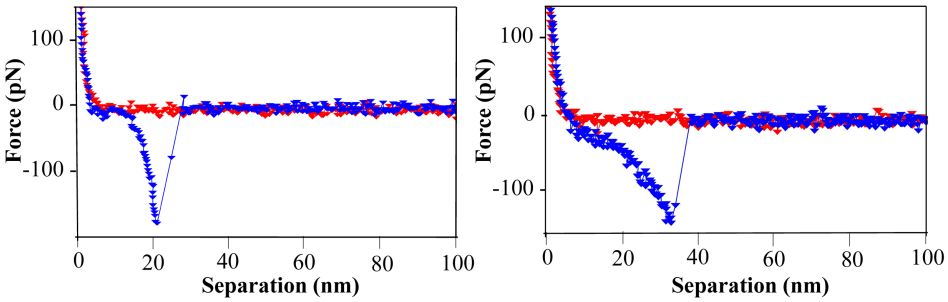


Figure 8. Specific adhesion force curve of an AFM tip modified with (a) methionine and (b) cysteine onpyrite surfaces. The red and blue traces indicate the signal upon approach and retraction from the surface, respectively.

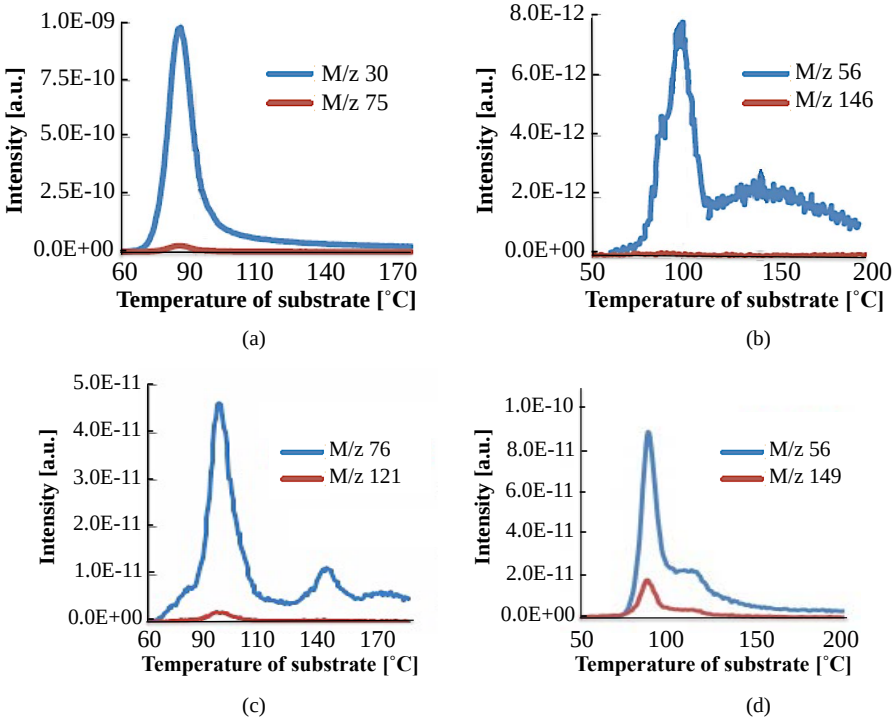


Figure 9. TDS spectra of (a) glycine; (b) lysine; (c) cysteine; and (d) methionine on pyrite (pH = 7.4). First and second peaks indicate the physisorption and chemisorption, respectively.

adsorption (physisorption), whereas the second peak refers to strong chemical adsorption (chemisorption). Beside the peak for glycine, two peaks appeared for lysine, cysteine, and methionine, indicating the presence of strong chemisorption of the molecules on the surface.

4. Discussion

Previous theoretical reports based on molecular dynamics simulations stated that the presence of non-stoichiometric sites with the molecular formula FeS_{2-x} resulting from sulfur vacancies that occurred due to changes in the electronic structure of pyrite enhanced the chemical reactivity and condensation of amino acids on pyrite surfaces [6]. Therefore, the pyrite surfaces provide higher probability of chemical bonding between Fe^{2+} of pyrite and $COOH^{-}$ group of the amino acid. Beside this theoretical assumption, it is worth noting that Yair Razvag *et al.* [13] studied the adsorption of several amino acids on inorganic surface. The authors mentioned that hydrophobic forces dominate the adhesion between the amino acid and the inorganic substrate. In addition, they concluded that the interplay between the nature of the residue of the amino acid (hydrophobic or charged) and its properties under certain environmental conditions with respect to the surface chemistry of the inorganic substrate controls the strength of the interaction between the amino acid and the surface. To our knowledge, this conclusion is not generally contradictive with our assumption when we chose to study glycine and other amino acids. This is because glycine, which does not have any side chain, showed no specific adhesion in the force measurement as well as no chemisorption in the TDS measurement (**Figure 9(a)**). We assume that glycine may be adsorbed onto pyrite surface via physical adsorption with the absence of chemical bonding between molecules, which refers to our AFM experiment where no specific event has been observed. However, those amino acids with side chains (lysine, cysteine, and methionine) showed chemisorption in the TDS experiments (**Figures 9(b)-(d)**), which was also proved with the specific interaction in AFM experiments, as previously shown in the force measurements section.

Moreover, according to many previous reports, sulfur-containing amino acids adsorb on pyrite. For instance, complex formation of cysteine and methionine with both iron and sulfur species of pyrite and the formation of FeS-R and FeS-S-R bridges was experimentally verified by J.A. Rojas-Chapana *et al.* [19] [20], which was also consistently observed in our AFM and TDS results. Furthermore, we assume that the characteristics of the side chain of the amino acids, *i.e.*, its charge (negative or positive) and polarity (polar or non-polar), may influence the adsorption and its interaction (or fails to interact) with pyrite. However, the exact mechanisms of how these amino acids adsorb to the pyrite surface are still under discussion, and more systematic studies are recommended for various amino acids on pyrite surface with different pH values and ionic strengths of the solution, which might affect the adsorption of the residues to the substrates.

5. Conclusions

In this study, we performed Raman and surface force analysis of pyrite, which is the most suitable catalyst for prebiotic chemistry, and observed that lysine, cysteine, and methionine amino acids with side chains showed chemical adsorption on the surface. The presence of non-stoichiometric sites, which resulted from sulfur vacancies due to changes in the electronic structure of pyrite, would enhance the chemical reactivity and condensation of amino acids on the pyrite surfaces.

Understanding the interactions at a single amino acid level is important for increasing our knowledge of peptide adsorption to inorganic surfaces. Pyrite may be a precursor of polymerization, and further experimental and theoretical analyses are required to confirm this hypothesis. Moreover, studying the interaction of amino acid having with various functional chains with pyrite is recommended in order to assess the capability of this mineral to adsorb and pattern biomolecules of increasing complexity. We believe that our findings will contribute to chemical evolution studies on the role of minerals which might have played during prebiotic chemistry and will thus further add to the complete knowledge of mineral-biomolecule interactions that is fundamental to assess its use in biotechnological applications as well.

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Supporting Information

TDS measurements

TDS measurements were performed to analyze molecules desorbed from natural pyrite by increasing their temperature. Before the measurements, natural pyrite was cleaned by deionized water under ultrasonication after being cut into substrates. The pyrite was heated to 350˚C in a vacuum during the experiment. By using a quadrupole mass spectrometer, the molecules desorbed from the pyrite were analyzed. The result showed that sulfur molecules desorbed from natural pyrite with heating to ≥280˚C, indicating the desorption of main sulfur-dimer (*m/z* 64) molecules and sulfur-monomer (*m/z* 32) molecules (**Figure S1**).

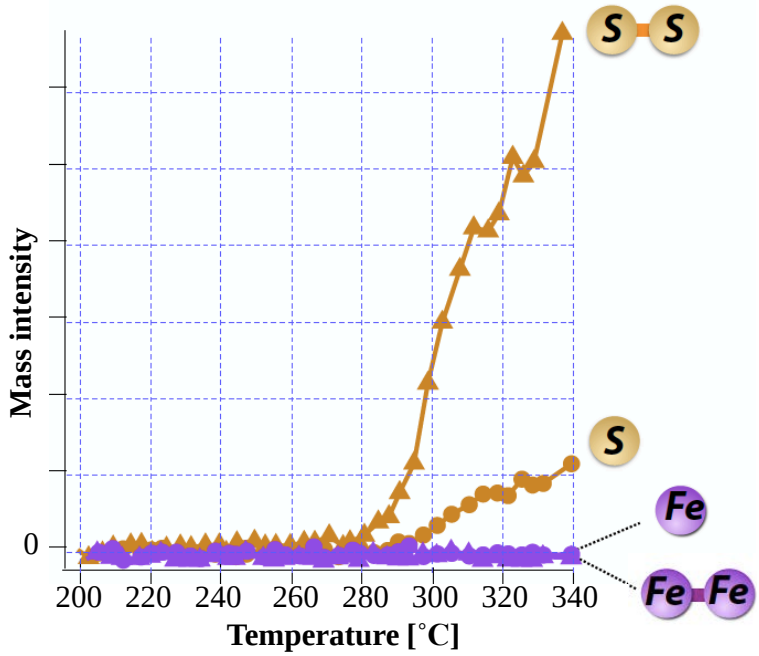

Figure S1. TDS spectra of sulfur-monomer (*m/z* 32) and sulfur-dimer (*m/z* 64) with temperature change of natural pyrite in vacuum. Sulfur-dimer peak is much higher than monomer peak. No significant desorption is observed for irons.