ELSEVIER

Biophysical Chemistry 91 (2001) 115–124

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Analysis of peptides synthesized in the presence of SAz-1 montmorillonite and Cu^{2+} exchanged hectorite

Timothy L. Porter[a,*], Michael P. Eastman[b], Edlin Bain[b], Shanadeen Begay[b]

[a]*Northern Arizona University, Department of Physics and Astronomy, Flagstaff, AZ 86011, USA*
[b]*Northern Arizona University, Department of Chemistry, Flagstaff, AZ 86011, USA*

Received 7 February 2001; received in revised form 29 March 2001; accepted 30 March 2001

Abstract

We have investigated the synthesis of oligopeptides containing glycine and tyrosine in the presence of the clay minerals montmorillonite (non-exchanged, SAz-1) and Cu^{2+} exchanged hectorite. In both cases, homopolymers of the two amino acids are formed, as are mixed peptides. In the case of Cu^{2+} hectorite, mixed oligopeptides up to trimers are detected in small amounts. For montmorillonite, heterogeneous oligopeptides up to hexamers are detected. Our experiments indicate montmorillonite is more effective in promoting oligopeptide formation than Cu^{2+} hectorite. Analysis of the oligopeptide sequences formed on the montmorillonite surfaces indicates preferential synthesis of certain Gly-Tyr sequences over others.

Keywords: Peptides; Montmorillonite; Hectorite; Glycine; Tyrosine

1. Introduction

The solid-phase synthesis of oligopeptides and oligonucleotides is an important area of study with regard to the origins of life on earth. Whether the first living, replicating molecular systems arose during the RNA world, or earlier in some environment based upon simpler genetic molecules (oligopeptides, peptidenucleic acids (PNAs), or other analogues of nucleotides) [1–3], long chain biological molecules were most likely required to produce viable, replicating systems. In order to form biopolymers of sufficient length to produce viable, replicating systems (approx. 30 mer or above [2]), a method of synthesis other than in aqueous solution is thought to be needed. In 1951, Bernal proposed that certain clay minerals might act to adsorb, concentrate and subsequently, catalyze the formation of such biopolymers. Using alternating cycles of heating and re-wetting, short peptides (up to approx. 6 mer) may be produced in the reaction of amino acids

* Corresponding author. Tel.: +1-520-523-2540; fax: +1-520-523-1371.

0301-4622/01/$ - see front matter
PII: S0301-4622(01)00159-4

in the presence of clay minerals [4–7]. Also, it has been demonstrated that clay minerals such as montmorillonite or hectorite may catalyze the formation of short oligonucleotides from the activated monomer [8–11].

While initial studies on activated nucleotides in the presence of montmorillonite produced only short chain polymers (10 mer), simulated pre-biotic protocols have been described in which much longer oligonucleotides are produced [12,13]. Certain clay minerals may also facilitate elongation of peptide oligomers. For example, it has been demonstrated that when the adsorption persistence of peptide oligomers increases with the number of residues in the oligomers, desorption of these oligomers from the mineral is subsequently reduced, and peptide elongation by activated monomer (even in dilute concentrations) is greatly facilitated [14]. Interestingly, on given clays, only certain peptides exhibit this elongation property, indicating that a variety of clay mineral types may have been involved in the template-directed synthesis of different peptides [14].

In this paper, we investigate the reactions of monomeric glycine and tyrosine with the clay minerals Cu^{2+} exchanged hectorite and SAz-1 montmorillonite. Only the monomers of these amino acids are used. While it has been suggested that peptide formation (primarily dimers) occurs more readily in evaporation reactions in the absence of any minerals [15–17], clay minerals can exhibit some selectivity with regard to the formation of higher oligomers [4,16,18]. Also, it has been demonstrated that the presence of certain specific amino acids (such as Gly) or certain peptides may enhance the formation of other peptides or higher oligomers of various amino acids [15].

2. Experimental

2.1. Materials

Cu(II) exchanged hectorite was prepared by stirring sodium hectorite (Rheox Corp.) in a solution of 0.5 M $CuSO_4$ for 24 h. The resulting material was washed with distilled water and then centrifuged until a negative test for SO_4^{2-} (Cu^{2+} clay) was obtained. SAz-1 was obtained from the Source Clay Minerals Repository (University of Missouri, Columbia). Thin hectorite or montmorillonite films were prepared by suspending the clay in deionized water, and casting onto suitable substrates. Amino acids were purchased from Aldrich Inc., and oligomer standards were purchased from Baychem, Inc.

2.2. Reaction method

Dried clay films were saturated with 30 mM (sum of all amino acids) solutions of glycine, tyrosine (L-optical isomer) or an equal combination of glycine (Gly) and tyrosine (Tyr). Some sonication of the tyrosine containing samples was used to aid in amino acid dispersion. Two samples of each reaction system were prepared. Results quoted are the average of the two samples. Each sample was then subjected to 10 alternate cycles consisting of heating to 85°C for 24 h, followed by rewetting with ultra-pure water for 24 h. A solution of 0.1 M calcium chloride was used to extract the organic material from the clays. These samples were centrifuged, then HPLC and mass analyzed in the LCQ system.

2.3. Analytical methods

Mass analysis was performed using a Finnigan LCQ system using atmospheric pressure ionization with an electrospray probe. The mass resolution of this system is currently 0.1–0.2 amu throughout its analysis range. This system is equipped with a narrow bore HPLC system permitting flow rates of 100–500 μl/min into the ESI-MS LCQ system. With this system, individual mass spectra are obtained every 5–10 s during the HPLC run, enabling direct mass analysis of each HPLC peak. All samples were run through a C-18 (Vydac 218TP52) column (for peptides). The elution gradient was: flow rate, 0.2 ml/min (narrow bore conditions); water (0.1% TFA)/ACN (0.085% TFA) 99:1 (30 min); water (0.1% TFA)/ACN (0.085% TFA) 25:75 (hold for 5 min); and recycle. HPLC and mass spectra were also obtained for standards including $Gly_{(2-6)}$

Table 1
Peptide standards used in the analysis of other peptides formed in the reactions of Gly and Tyr with montmorillonite and hectorite

Gly standards	Tyr standards	Gly-Tyr standards
Gly_2	Tyr	H-Gly-Tyr-OH
Gly_3	Tyr_2	H-Tyr-Gly-OH
Gly_4	Tyr_3	H-Gly-Gly-Tyr-OH
Gly_5		H-Gly-Tyr-Gly-OH
Gly_6		H-Tyr-Gly-Gly-OH

oligomers standards, $Tyr_{(1-3)}$ oligomers standards and GlyTyr or Gly_2Tyr oligomers standards (Table 1).

2.4. LC-MS spectra

LC-MS spectra of the peptides extracted from the various clays allows for nearly unambiguous determination of the peptides formed in the reaction of the clay minerals with the amino acids. In this study, standard reverse-phase HPLC is combined with electrospray mass spectrometry (ES-MS). After passing through the C-18 column, eluent is injected directly into the ES-MS. Mass spectra are acquired approximately every 10 s during the entire HPLC run, and the mass spectra obtained are synchronized in time with the HPLC spectra. Individual mass spectra at any given time, such as at each HPLC peak, may be analyzed. Alternately, MS ion counts at a specific mass may be displayed as a function of time (total ion current, or TIC method). The latter method is especially useful for investigating the separation of individual peptides in the reverse-phase column.

3. Results and discussion

Combined LC-MS spectra of various peptide oligomer standards are presented in Fig. 1. In Fig. 1a, a HPLC spectrum of standards Gly_{2-6} is shown, along with the corresponding mass spectrum total ion current for the five Gly oligomers. The HPLC spectrum and TIC spectrum for various Tyr-containing peptides is shown in Fig. 1b. As can be seen from Fig. 1a, HPLC separation of Gly homopeptides under the conditions used is not complete, with a great deal of overlap between each successive oligomer. The ES-MS TIC spectrum shows the total ion current for each individual Gly oligomer, with the mass resolution window set to 0.1 amu (i.e. for Gly_2 the ion current is plotted for ions between mass 133.0 and 133.1 amu, for Gly_3 the window is 190–190.1 amu and so on). Little or no separation of Gly oligomers under this HPLC protocol is expected, however, it is clear from the combined LC-MS spectra that longer Gly oligomers are eluting slightly more slowly from this column than shorter oligomers.

In Fig. 1b, the combined LC-MS spectra for Tyr_2, Tyr_3, TyrGly and $TyrGly_2$ standards are shown. Here, the individual HPLC peaks match up perfectly with the mass spectral peaks for the given oligomers. Also, it is interesting to note that the addition of Gly to Tyr alters the HPLC column passage of the combined oligomer significantly, which might not be expected from first order hydrophobicity considerations of these peptides. We also ran TyrGly and $TyrGly_2$ oligomer standards for the possible sequences of these peptides. From the HPLC spectra, a single peak with one or two shoulders was observed. For the Tyr-Gly dimers (H-Tyr-Gly-OH and H-Gly-Tyr-OH), mass spectra showed a slight separation only (the H-Tyr-Gly-OH dimer contains the fragment $m = 164$, while the H-Gly-Tyr-OH fragment contains fragment $m = 182$, see Section 3.4 on sequencing below). For the trimer standards, we were unable to determine any measurable separation between the three oligomers using mass analysis.

3.1. Glycine oligomers

The production of glycine oligomers from Cu^{2+} hectorite exposed to Gly monomer only, Cu^{2+} hectorite exposed to Gly and Tyr monomer, SAz montmorillonite exposed to Gly monomer and SAz montmorillonite exposed to Gly and Tyr monomer is summarized in Table 2. The relative amount of each oligomer produced is determined by measuring the total ion current produced by that particular oligomer, i.e. the total ion current

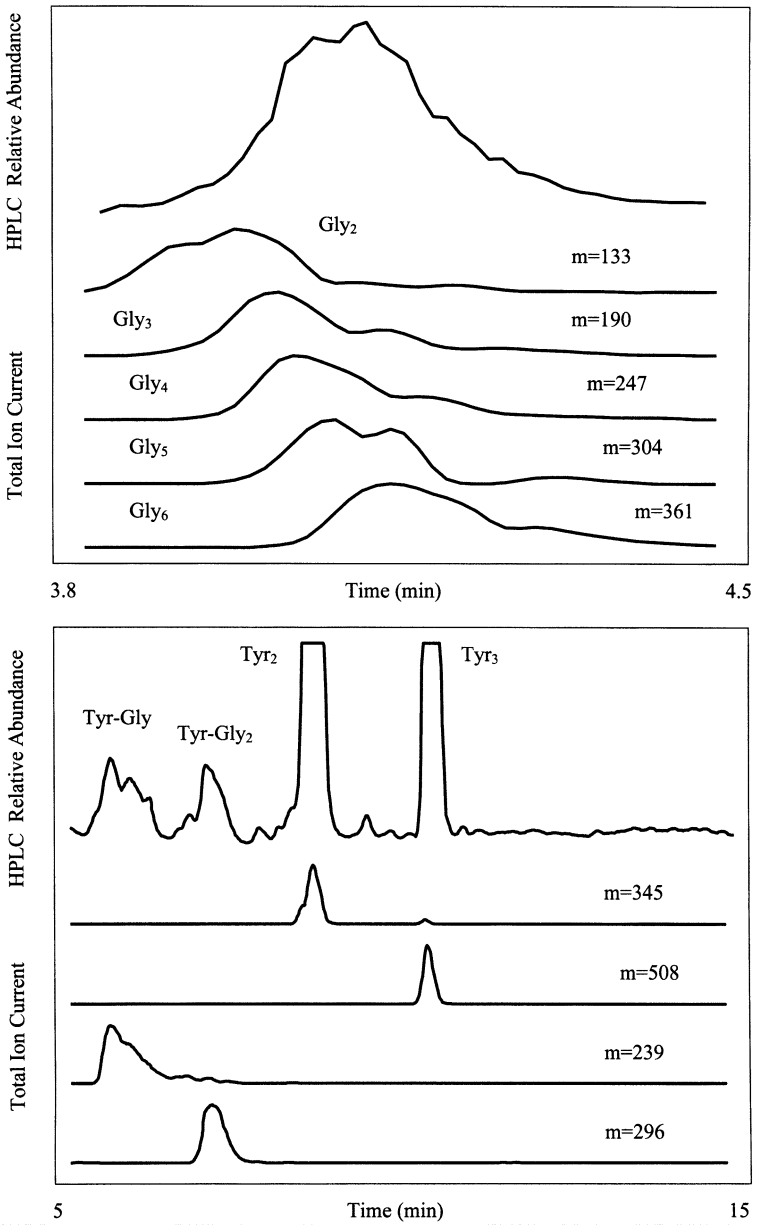

Fig. 1. (Upper window) HPLC spectrum (top), and mass spectrum total ion current (TIC) data (bottom) for Gly oligomers. While HPLC separation of these Gly oligomers is poor, the time-resolved mass spectra clearly differentiate between the individual oligomers. (Lower window) HPLC spectrum and TIC spectra for various Tyr-Gly oligomers. Here, HPLC separation of the oligomers is good, owing to the presence of the hydrophobic Tyr units.

at a particular molecular weight ± 0.1 amu. While it is possible to compare absolute yields from one sample to the next, our discussion will focus primarily on the relative oligomer yields within a given family of oligomers. The numbers appearing in Table 2 and Table 4 are percentage yields

Table 2
Relative percentages of Gly oligomers formed in the reactions of Gly only with SAz montmorillonite and Cu^{2+} hectorite, as well as Gly and Tyr reacted with the same two clays[a]

	SAz-1 Montmorillonite Gly	Cu^{2+} Hectorite Gly	Cu^{2+} Hectorite Gly-Tyr	SAz-1 Montmorillonite Gly-Tyr
Gly_2	6.70	4.10	0.14	0.15
Gly_3	1.90	0.58	0.48	0.02
Gly_4	0.56	0.37	0.25	0.06
Gly_5	0.40	0.12	0.19	0.13
Gly_6	0.28	0.07	0.09	0.08

[a]All numbers are $\times 10^{-4}$. For Cu^{2+} hectorite reacted with Gly-Tyr, we note a depletion of Gly_2 with respect to Cu^{2+} hectorite reacted with Gly only. Similarly, in the montmorillonite sample reacted with Gly-Tyr, depletion of Gly_{2-4} occurs.

($\times 10^{-4}$) based on the initial amount of amino acid reacted (or, 10^{-6} actual yield). The relative abundances of Gly_{2-6} oligomers in the Cu^{2+} hectorite samples exposed to glycine only agrees well with previous data obtained by us on this reaction system [7]. For this system, Gly oligomers up to 6 monomer units are produced, with Gly_2 being the most abundant product. Higher oligomers are produced in decreasing amounts up to Gly_6. In all samples both Gly monomer and cyc(Gly_2) (or diketopiperazine, DKP) are also detected (but not listed). For the SAz montmorillonite sample exposed to Gly monomer, we note similar activity with somewhat higher yields for all of the products Gly_{2-6}.

For the clays exposed to both Gly and Tyr monomers, the production of Gly_{2-6} oligomers does not follow the same pattern. For the Cu^{2+} hectorite samples, the order of relative abundance in Gly oligomers is Gly_3, Gly_4, Gly_5, Gly_2 and Gly_6. For the SAz-1 samples, the order of Gly oligomer production is Gly_2, Gly_5, Gly_6, Gly_4 and Gly_3. One general trend is apparent in these samples. In the mixed Gly-Tyr samples, there is some apparent depletion of shorter Gly oligomers. For example, in the Cu^{2+} hectorite samples, depletion is occurring for Gly_2 oligomers. In the SAz-1 sample, depletion is occurring for Gly_2, Gly_3 and Gly_4 oligomers. The depletion of Gly_3 and Gly_4 oligomers in the SAz-1 samples is nearly 100%. We believe that depletion of these particular Gly oligomers is due to reaction with Tyr to produce mixed Gly-Tyr peptides (discussed below).

3.2. Tyrosine oligomers

Table 3 lists the relative production of Tyr homopeptides for the hectorite and montmorillonite clays. For the clays exposed to Tyr monomer only, we see little peptide formation. In the case of Cu^{2+} hectorite, no detectable Tyr peptides are produced, while for SAz-1 montmorillonite, only Tyr_2 is produced (trace). When the same clays are

Table 3
Production of Tyr homopolymers from Cu^{2+} hectorite and SAz montmorillonite reacted with Gly, Tyr, or Gly-Tyr[a]

	SAz-1 Montmorillonite Tyr	Cu^{2+} Hectorite Tyr	Cu^{2+} Hectorite Gly-Tyr	SAz-1 Montmorillonite Gly-Tyr
Tyr_2	Trace	0.00	0.09	2.5
Tyr_3	0.00	0.00	0.00	1.3

[a]The synthesis of Tyr homopolymers is enhanced by the addition of Gly monomer. Tyr oligomers greater than 3 units were not detected.

reacted with both Gly and Tyr monomer, the resulting formation of Tyr oligomers is enhanced somewhat. The SAz-1 samples show the presence of both Tyr_2 and Tyr_3, but no higher Tyr oligomers. The Cu^{2+} hectorite samples show a small amount of Tyr_2 oligomer synthesis. It is possible that the presence of Gly (and possibly Gly peptides) is enhancing the formation of Tyr peptides in the samples containing both amino acids. A similar catalytic effect involving Gly in the presence of Val, Leu and Lys has previously been reported [15]. In this study, the addition of Gly to pre-biotic reaction mixtures utilizing a salt-induced peptide formation (SIPF) protocol was found to enhance the formation of Val_2, Leu_2 and Lys_2. The addition of Gly_2 as a catalyst produced even greater yields of these oligomers in some cases.

Table 4
Synthesis of mixed Gly-Tyr oligomers for the clays Cu^{2+} hectorite and SAz montmorillonite[a]

	Cu^{2+} Hectorite Gly-Tyr	SAz-1 Montmorillonite Gly-Tyr
TyrGly	0.5	3.0
$TyrGly_2$	0.22	2.3
Tyr_2Gly	0.09	1.3
$TyrGly_3$	0.00	1.9
$TyrGly_4$	0.09	1.4
Tyr_2Gly_2	0.00	1.2
$TyrGly_5$	0.00	1.0
Tyr_3Gly	0.00	0.00

[a]For the montmorillonite sample, greater numbers of oligomers are detected out to 6 amino acid units. For the hectorite clay, only small amounts of mixed dimers and trimers are formed.

3.3. Glycine-Tyrosine oligomers

In Table 4 the relative abundances of mixed Gly-Tyr oligomers are shown. For the Cu^{2+} hectorite sample, a moderate amount of TyrGly is present, as is $TyrGly_2$. Some Tyr_2Gly is also present in this sample. For the Cu^{2+} hectorite sample with both Gly and Tyr, this abundance of $TyrGly_2$ may help explain the observed depletion of Gly_2 (Table 2). For the SAz-1 sample, larger amounts of TyrGly, $TyrGly_2$ and Tyr_2Gly are present.

For mixed Gly-Tyr oligomers larger than 3 monomer units, the SAz-1 clay samples are very active in comparison to the Cu^{2+} hectorite sample. In the SAz-1 clay, relatively large amounts of both $TyrGly_3$ and $TyrGly_4$ are present, as is Tyr_2Gly_2. Mass spectra indicate a small amount of $TyrGly_5$ also. In contrast, the Cu^{2+} hectorite samples show no evidence of any mixed oligomer formation larger than 3 monomer units. Also, the presence of large amounts of $TyrGly_3$ and $TyrGly_4$ in the montmorillonite samples reacted with both Gly and Tyr helps to explain the near complete depletion of Gly_3 and Gly_4 homopeptides (Table 2). It is clear that while both the Cu^{2+} hectorite and SAz montmorillonite clays are reasonably efficient at producing Gly homopeptides, the montmorillonite clay is considerably more active than the hectorite in producing mixed Gly-Tyr oligomers, especially those containing only 1 Tyr unit.

It is interesting to compare these results with previous studies concerning the selectivity of various clay minerals to peptide or oligopeptide formation. In studies involving Ca-montmorillonite and Na-hectorite, the formation of higher peptides of glycine and alanine (5 or 6 monomer units) was enhanced with respect to formation of these oligomers in the absence of the clay [16]. These reactions were carried out under somewhat different conditions, involving the use of copper(II) chloride and NaCl solutions in addition to the clay minerals. Also, the use of the peptide dimers as a starting point was utilized in most of the reactions. The reasoning behind this reaction methodology is that peptide dimers are formed more efficiently in a SIPF (salt-induced peptide formation) reaction than in a simple reaction involving amino acid monomers and clay minerals. The SIPF reaction (involving dehydration of the Cu(II) and NaCl plus monomer solution) is proposed to produce larger levels of peptide dimers, which may then be elongated in reactions with the clay minerals and additional amino acids [15–17,19]. In addition to the generalized enhancement of the formation of longer oligomers with the clay minerals present, some

clay selectivity was also reported. For example, in reactions involving glycine and diglycine with these clays, it was reported that Gly_6 was formed preferentially over Gly_5 in the presence of Ca-montmorillonite, while the reverse was true when the same reaction was carried out using hectorite [16]. Also, for reactions involving alanine and dialanine, the hectorite catalyst produced a greatly enhanced yield of Ala_3 with respect to the reaction in the presence of montmorillonite [16]. Other clay selectivity effects for Gly and Ala (dimer only) reactions were also reported [16].

In another study [18], mixtures of amino acids were reacted with Ca-hectorite, alumina and silica in a pre-biotic temperature and hydration cycling scheme. Peptide bond formation for heterogeneous oligopeptides up to 3 monomer units was reported. The efficiencies for catalyzing peptide bond formation increased in the order hectorite, silica and alumina. Only alumina catalyzed all peptide bond reactions that were identified using HPLC.

3.4. Peptide sequencing

When the molecular mass of a peptide is known, for example $Gly_2Tyr = 296$, we may use the fragmentation effect of ES-MS to learn the sequence of the peptide. Backbone (peptide bond) cleavages in the mass spectrometer generate two types of ions. Acylium ions are produced when the charge is retained on the N-terminal side of the peptide, while protonated peptides are produced when the charge is retained on the C-terminal side of the peptide. These ion fragments are known as b_n and y_n fragments, respectively. In straightforward cases, all possible fragments are produced using ES-MS. This means that for a given peptide sequence, two unique sets of fragment ions are produced (the b_n and y_n sets). In Table 5a, the 'predicted' fragments for a given peptide are listed. Comparison of these predicted fragments with experimentally observed peptide fragments allows for a given peptide mass to be assigned to a unique peptide sequence.

In Table 5b we list the predicted and experimentally observed fragments corresponding to the peptide masses GlyTyr (239) Gly_2Tyr (296) and Tyr_2Gly (402) for the SAz montmorillonite sample reacted with Gly and Tyr monomer (we do not include a table for fragments produced in the Cu^{2+} hectorite sample. In the Cu^{2+} hectorite sample, the amount of Gly-Tyr combination peptides was small enough so that ambiguous results were obtained. See further explanation below). For GlyTyr, two sequences are possible: H-Gly-Tyr-OH; and H-Tyr-Gly-OH. For the Gly_2Tyr mass, three individual sequences are possible: H-Gly-Gly-Tyr-OH; H-Gly-Tyr-Gly-OH; and H-Tyr-Gly-Gly-OH. Also, three possible sequences are possible for Tyr_2Gly: H-Tyr-Tyr-Gly-OH; H-Tyr-Gly-Tyr-OH; and H-Gly-Tyr-Tyr-OH. As a test of this analysis process we examined the fragments produced by two standards, H-Tyr-Gly-OH and H-Gly-Gly-Tyr-OH. In the case of the dimer, fragments were found at masses 164, 221 and 239, but none at mass 239 (we did not look at masses below 100). From Table 5, we see that the missing fragment at mass 239 is sufficient to identify the sequence of this dimer as H-Tyr-Gly-OH. For the trimer, fragments at mass 133, 164 and 221 were absent, while all of the fragments for the sequence H-Gly-Gly-Tyr-OH were present.

For the experiments using SAz-1 reacted with Gly and Tyr monomer, an asterisk beside a given predicted fragment indicates that that fragment is observed in the mass spectrum for the given peptide mass. Our criteria for a given peptide fragment to be counted as 'there' is that it appear at the correct time, i.e. appear at the same time during the scan that the parent peptide appears and that it have an intensity of at least double the mass spectral background counts. We note that using this criterion, certain fragments we list as 'missing' may actually be present, but in amounts too small to reliably detect.

For the mixed dimers produced in the SAz-1 sample, fragments at mass 182 are found to be missing (masses below 100 were not measured). This indicates that the dimer formed is H-Tyr-Gly-OH. Also, owing to the sequences of trimers obtained (see below), this peptide appears to no longer elongate by addition of single amino acids.

Table 5
(a) Formulas for prediction of mass spectrometer fragments during the analysis of peptides. Two series (b_n and y_n) are produced, depending on the peptide end on which cleavage occurs. (b) Predicted peptide fragments for Gly-Tyr dimers and trimers for the possible sequences for each[a]

A Predicted peptide fragments			
b_1	$M_{residue} + H$	y_1	$M_{residue} + 19$
b_2	$M_{residue} + b_1$	y_2	$M_{residue} + y_1$
b_{n-1}	$MH^+ - 18 - M_{residue}$	y_{n-1}	$MH^+ - M_{residue}$

B Fragment masses for selected peptides * fragment is selected in mass spectrum

H-Tyr-Gly-OH	H-Gly-Tyr-OH	
$b_1 = 164$*	$b_1 = 58$	
$b_2 = 221$*	$b_2 = 221$*	
$y_1 = 76$	$y_1 = 182$	
$y_2 = 239$*	$y_2 = 239$*	
H-Gly-Gly-Tyr-OH	H-Gly-Tyr-Gly-OH	H-Tyr-Gly-Gly-OH
$b_1 = 58$	$b_1 = 58$	$b_1 = 164$
$b_2 = 115$*	$b_2 = 221$	$b_2 = 221$
$b_3 = 278$*	$b_3 = 278$*	$b_3 = 278$*
$y_1 = 182$*	$y_1 = 76$	$y_1 = 76$
$y_2 = 239$*	$y_2 = 239$*	$y_2 = 133$*
$y_3 = 296$*	$y_3 = 296$*	$y_3 = 296$*
H-Tyr-Tyr-Gly-OH	H-Tyr-Gly-Tyr-OH	H-Gly-Tyr-Tyr-OH
$b_1 = 164$	$b_1 = 164$	$b_1 = 58$
$b_2 = 327$*	$b_2 = 221$*	$b_2 = 221$*
$b_3 = 384$*	$b_3 = 384$*	$b_3 = 384$*
$y_1 = 76$	$y_1 = 182$*	$y_1 = 182$*
$y_2 = 259$	$y_2 = 259$	$y_2 = 345$*
$y_3 = 402$*	$y_3 = 402$*	$y_3 = 402$*

[a]An asterisk next to a given mass indicates that that particular fragment is detected.

The absence of the dimer H-Gly-Tyr-OH does not necessarily mean it is not formed, however. One assumption might be that any H-Gly-Tyr-OH that is formed reacts further to form the trimer H-Gly-Tyr-Tyr-OH (which is seen here). If initial adsorption of an amino acid (or higher peptide) occurs by interaction of the positively charged amino group of the amino acid with negative sites on the clay edges, such as Mg–O or Al–O sites, these sites may become the 'anchors' for the growing peptide chains. The carboxyl group at the free end of the chain thus becomes the site for chain elongation to occur. The carboxyl group of the growing peptide may interact with Si–O groups (also possibly at clay edges), resulting in activation that subsequently, facilitates elongation of the chain via a condensation reaction [20–23]. Thus, we do not expect a dimer such as H-Gly-Tyr-OH to form H-Gly-Gly-Tyr-OH through an elongation reaction on the clay surface.

For the mixed trimers formed in the reaction of SAz-1 with glycine and tyrosine, only two sequences are detected. These are H-Gly-Gly-Tyr-OH and H-Gly-Tyr-Tyr-OH. One scenario for the formation of the H-Gly-Gly-Tyr-OH peptide involves the simple reaction of diglycine with Tyr. Since the homopeptide Gly_2 is strongly depleted in this sample (Table 1), this is an indication that nearly all H-Gly-Gly-OH formed on the clay surface reacts further to form the trimer H-Gly-Gly-Tyr-OH. Furthermore, noting the depletion of the higher glycine homopeptides Gly_3 and Gly_4, we may postulate that these peptides also react quickly with Tyr to form H-Gly-Gly-Gly-Tyr-OH

and H-Gly-Gly-Gly-Gly-Tyr-OH, respectively. The other trimer detected in this reaction, H-Gly-Tyr-Tyr-OH, may be formed from addition of Tyr to the dimer H-Gly-Tyr-OH (as discussed above), or possibly from the addition of Tyr_2 to Gly adsorbed on the clay surface. We note that these are only possible explanations for the observed products, other complex reactions such as sequence inversions or chain elongation via reactions involving diketopiperazines may be occurring.

For the reaction of Cu^{2+} hectorite with glycine and tyrosine, the production of mixed peptides is greatly reduced. The small amounts of the mixed peptides that are produced makes identification of their respective fragments more difficult. There is evidence that both dimers are produced, i.e. H-Gly-Tyr-OH and H-Tyr-Gly-OH, as well as all three possible trimers involving Gly_2Tyr. For the trimers involving Tyr_2Gly, we were not able to detect fragments at masses 221, 259 or 327. This would indicate that none of these particular trimers are formed, however, the amounts of these products are so small that we may simply not be able to reliably detect them.

These results point to generally strong differences in the pre-biotic synthesis of peptide chains, owing primarily to differences in the minerals catalyzing these reactions. In the case of montmorillonite, e.g. it seems likely that all possible Gly_xTyr_y combination peptides may be catalyzed, but a large degree of selectivity is occurring with regard to the precise amino acid sequence produced within a given family of Gly_xTyr_y peptides. The abundance and spatial distribution of reactive sites near the clay edges would seem to be the key element in this selective process. These sites might determine how the growing peptide is physically anchored or adsorbed to the substrate (which is critical in preventing hydrolysis during these reactions), how it is oriented and whether or not the growing end of the chain is at or near a surface site where it may become activated and thus elongated. The preferred reactions will be those in which the mineral structure, geometry and distribution of reactive sites and amino acid or peptide size, shape and chemical properties all interact favorably.

Acknowledgements

This work was supported by the National Science Foundation (DMR 0071672).

References

[1] G.F. Joyce, L.E. Orgel, Prospects for Understanding the Origin of the RNA World, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1993.
[2] J.W. Szostak, A.D. Ellington, The RNA World, Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y, 1993.
[3] R. Liu, Polymerization of beta-amino acids in aqueous solution Orig. Life Evol. Biosph. 28 (1998) 47–60.
[4] J. Bujdàk, B.M. Rode, The effect of smectite composition on the catalysis of peptide bond formation J. Mol. Evol. 43 (1996) 326–333.
[5] N. Lahav, D. White, S. Chang, Peptide formation in the pre-biotic era: thermal condensation of glycine in fluctuating clay environments Science 201 (1978) 67–69.
[6] J.G. Lawless, N. Levi, The role of metal ions in chemical evolution: polymerization of alanine and glycine in a cation-exchanged clay environment J. Mol. Evol. 13 (1979) 281–286.
[7] T.L. Porter, M.P. Eastman, L.B. Price, R.F. Shand, Site specific prebiotic polymerization of glycine on the surface of cu(ii)-exchanged hectorite J. Mol. Evol. 47 (1998) 373–377.
[8] J.P. Ferris, G. Ertem, Oligomerization of ribonulceotides on montmorillonite: reaction of the 5′-phosphorimidazolide of adenosine Science 257 (1992) 1387–1389.
[9] J.P. Ferris, G. Ertem, Oligomerization reactions of ribonucleotides: the reaction of the 5′-phosphorimidazolide of nucleosides on montmorillonite and other minerals Orig. Life Evol. Biosph. 22 (1992) 369–381.
[10] J.P. Ferris, G. Ertem, Montmorillonite catalysis of rna oligomer formation in aqueous solution. A model for the pre-biotic formation of RNA J. Am. Chem. Soc. 115 (1993) 12270–12275.
[11] T.L. Porter, R. Whitehorse, M.P. Eastman, E.D. Bain, Studies on the reaction of the 5′-phosphorimidazolide of adenosine with cu(ii)-exchanged hectorite Appl. Phys. Lett. 75 (1999) 2674–2676.
[12] J.P. Ferris, A.R.J. Hill, R. Liu, L.E. Orgel, Synthesis of long prebiotic oligomers on mineral surfaces Nature 381 (1996) 59–61.
[13] L.E. Orgel, Polymerization on the rocks: theoretical introduction Orig. Life Evol. Biosph. 28 (1998) 227–234.
[14] A.R. Hill, C. Bohler, L.E. Orgel, Polymerization on the rocks: negatively charged alpha amino acids Orig. Life Evol. Biosph. 28 (1998) 235–243.
[15] Y. Suwannachot, B.M. Rode, Mutual amino acid catalysis in slat-induced peptide formation supports this mechanisms role in pre-biotic peptide evolution Orig. Life Evol. Biosph. 29 (1999) 463–471.

[16] B.M. Rode, H.L. Son, Y. Suwannachot, J. Bujdak, The combination of salt induced peptide formation reaction and clay catalysis: a way to higher peptides under primitive earth conditions Orig. Life Evol. Biosph. 29 (1999) 273–286.

[17] Y. Suwannachot, B.M. Rode, Catalysis of dialanine formation by glycine in the salt-induced peptide formation reaction Orig. Life Evol. Biophs. 28 (1998) 79–90.

[18] J. Bujdak, B.M. Rode, Silica, alumina and clay catalyzed peptide bond formation: enhanced efficiency of alumina catalyst Orig. Life Evol. Biosph. 29 (1999) 451–461.

[19] J. Bujdák, A. Eder, Y. Yongyai, K. Faybíková, B.M. Rode, Investigation on the mechanism of peptide chain prolongation on montmorillonite J. Inorg. Biochem. 61 (1996) 69–78.

[20] D. Jewett, J. Lawless, Detection of activated acyl groups formed by heating carboxylic acids with silica Naturwis 68 (1981) 570–571.

[21] D.H. White, R.M. Kennedy, J. Macklin, Acyl silicates and acyl aluminates as activated intermediates in peptide formation on clays Orig. Life 14 (1984) 273–278.

[22] V.A. Basiuk, T.Y. Gromovoy, V.G. Glovaty, A.M. Glukhoy, Mechanism of amino acid polycondensation on silica and alumina surface Orig. Life Evol. Biosph. 20 (1991) 483–498.

[23] J.R. Collins, G.H. Loew, B.T. Luke, Theoretical investigation of the role of clay edges in pre-biotic peptide bond formation Orig. Life Evol. Biosph. 18 (1988) 107–119.