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PREBIOTIC CHEMISTRY

The Role of Submarine Hydrothermal Systems in the Synthesis of Amino Acids

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Abstract There is little consensus regarding the plausibility of organic synthesis in submarine hydrothermal systems (SHSs) and its possible relevance to the origin of life. The primary reason for the persistence of this debate is that most experimental high temperature and high-pressure organic synthesis studies have neglected important geochemical constraints with respect to source material composition. We report here the results of experiments exploring the potential for amino acid synthesis at high temperature from synthetic seawater solutions of varying composition. The synthesis of amino acids was examined as a function of temperature, heating time, starting material composition and concentration. Using very favorable reactant conditions (high concentrations of reactive, reduced species), small amounts of a limited set of amino acids are generated at moderate temperature conditions (~125–175°C) over short heating times of a few days, but even these products are significantly decomposed after exposure times of approximately 1 week. The high concentration dependence observed for these synthetic reactions are demonstrated by the fact that a 10-fold drop in concentration results in orders of magnitude lower yields of amino acids. There may be other synthetic mechanisms not studied herein that merit investigation, but the results are likely to be similar. We conclude that although amino acids can be generated from simple likely environmentally available precursors under SHS conditions, the equilibrium at high temperatures characteristic of SHSs favors net amino acid degradation rather than synthesis, and that synthesis at lower temperatures may be more favorable.

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Introduction

Submarine hydrothermal systems (SHSs) were first suggested as possible environments for the origin of life soon after their discovery at the Galapagos spreading center (Corliss et al. 1981). This idea has been bolstered by the presence of flourishing hydrothermal vent ecosystems, controversial interpretations of molecular phylogenic data that the last common ancestor of all modern organisms was thermophilic (e.g. Brochier and Philippe 2002), and the idea that subsurface environments would have offered protection for life to emerge on a tumultuous early Earth (Maher and Stevenson 1988). Theoretical calculations proposing the synthesis of organic compounds under simulated SHS conditions support this idea (Shock 1992a), and have been extrapolated to the possible origin of life on extraterrestrial bodies including Mars (Bock and Goode 1996) and Europa (Chyba and Phillips 2001). This argument is countered by studies demonstrating the low stability of biomolecules at high temperatures and extreme pH values (e.g. Bernhardt et al. 1984; White 1984; Miller and Bada 1988; Bada et al. 1995; Ito et al. 2006). Therefore one central question regarding prebiotic organic molecule inventories is whether SHSs are net creators or destroyers of organic compounds, which is highly dependent upon the conditions within these systems.

Amino acids have been synthesized in laboratory simulations with yields generally no greater than a few percent (Table 1) using reactive starting compounds subjected to high temperatures and pressures. Most aqueous studies use unrealistically short heating times on the order of minutes to hours while in reality, residence times in axial hydrothermal environments range from years (Coumou et al. 2008; Kadko and Butterfield 1998) to decades (Turekian and Cochran 1986), while those in lower temperature off-axis diffuse flow systems may be on the order of thousands of years (Johnson and Pruis 2003). Because these residence times are impractical to model experimentally, the effect of long residence times in SHSs must be extrapolated from shorter timescale experiments. Most experimental studies have used unrealistically high starting concentrations of reduced reactive compounds such as NH_3, HCN, CH_4, and HCHO (e.g. Islam et al. 2001). Although high starting concentrations are often necessary to detect compounds produced in very low yields, the initial and equilibrium reactant concentrations are important constraints as there is likely a reactant concentration threshold, exceeded in most studies, below which little or no detectable net synthesis occurs.

Absolute upper limits of oceanic carbon and nitrogen species in the early Archean oceans may be estimated by assuming that all of the Earth's carbon from crustal and atmospheric reservoirs was dissolved into oceans of the present volume ($\sim 10^{21}$ l). This would give concentrations of 1-carbon compounds in the primitive ocean of approximately 1 M and a maximum concentrations of oceanic single-nitrogen atom species of ~0.2 M (Schwartz 1981). More realistic estimates of total primitive oceanic carbon concentrations have been estimated as ~130 mM, mostly in the form of bicarbonate, based on the amount of CO_2 needed to keep surface water in the liquid state due to the decreased luminosity of the early sun (Morse and Mackenzie 1998). Of this carbon reservoir, reduced reactive species probably constituted only a small percentage in the early oceans. For instance, upper limit concentrations of hydrogen cyanide (HCN) in the primitive oceans have been estimated as $\sim 2 \times 10^{-6}$ M (at pH 8 at 0°C; Miyakawa et al. 2002). Ammonia concentrations have been estimated $\sim 2 \times 10^{-7}$ M (Summers 1999), and total prebiotic oceanic nitrogen

Table 1 Amino acid yields and synthesis conditions for aqueous hydrothermal synthesis experiments. Yields shown are based on input nitrogen

	Principle amino acids products		Starting materials		T (°C)	Time (hours)
	Percent yield	Amino acids formed	Carbon species	Nitrogen species		
Oró et al. 1959	~7.1%	Gly >> β-Ala > Ala	HCHO (0.25 M)	$NH_2OH{\cdot}HCl$ (0.25 M)	100°C	60
Lowe et al. 1963 (1)	~0.45%	Gly >> Ser > Asp	HCN (1.5 M)	NH_4OH (1.5 M)	90°C	18
Wolman et al. 1971 (2)	~0.019%	Gly >> Glu > Ala	HCHO (10 M)	NH_4OH (3.7 M)	185°C	8
Kamaluddin et al. 1979 (3)	~2.6%	Gly > Ser > Asp > β-Ala	HCHO (0.3 M)	NH_2OH (0.05 M)	105°C	50 days
Hennet et al. 1992 (4)	~2.4%	Gly >> Asp > Ala	HCHO (0.18 M) KCN (0.19 M)	NH_4Cl (0.23 M)	150°C	25
Yanagawa and Kobayashi 1992 (5)	~0.01%	Gly > Sar > Ala	CH_4 (~1.6 M)	NH_4Cl (50 mM)	325°C	12
Marshall 1994 (6)	~0.004%	Gly > Ala > Asp	HCHO (0.75 M)	NH_4HCO_3 (0.75 M)	210°C	2
Islam et al. 2001 (7)	~13%	Gly > Sar > Ala	HCHO (0.1 M) KCN (0.1 M)	NH_4HCO_3 (0.05 M)	250°C	2 mins

(1) Maximum yields reported, experiment 6

(2) Results using distilled reagents under N_2 are reported. Experiment modeled after Fox and Windsor (1970)

(3) Similar to results from Hatanaka and Egami (1977) using identical modified sea medium (composition detailed in paper; pH=5.58)

(4) Data reported for the PPM buffered experiment. Solution contained ~0.08 M HCl, 0.05 M NaHS, and 0.1 mg of platinum powder included as a sink for O_2. Experiments run at pH~7 using a titatium oxide autoclave liner

(5) Recovery based on input NH_4Cl. Solution was heated in the presence of nitrogen at a pH of ~3.6 with HCl and contained 2 mM $Fe(NH_4)_2(SO_4)_2$, 0.6 mM $MnCl_2$ 0.1 mM $ZnCl_2$ 0.1 mM $CuCl_2$, 20 mM $CaCl_2$, 0.1 mM $BaCl_2$ and 50 mM NH_4Cl

(6) Results from experiment 5 reported, however the addition of 0.75 M NaCN resulted in ~15% yields

(7) Only very short heating times (2–5 min) were detailed in this study. Runs were conducted at 25 Mpa at a pH of ~10.7 within a Hastelloy reactor. Results are similar to those from Marshall (1994) with NaCN

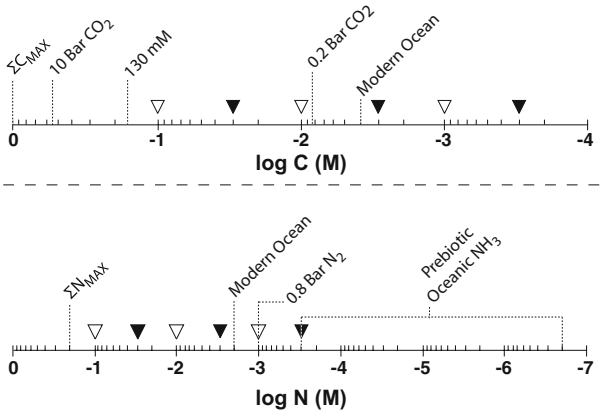


Fig. 1 Total carbon and nitrogen concentrations for ammonium formate (▽ =100, 10, 1 mM) and synthetic seawater solution (▼ =30, 3, 0.3mM) experiments shown relative to other estimates for the primitive oceans. Upper limit concentrations (ΣC_{MAX}, ΣN_{MAX}) determined by Schwartz (1981), prebiotic oceanic ΣCO_2 estimate (130 mM) from Morse and Mackenzie (1998), and prebiotic [NH_3] estimates from Summers (1999). The estimates based on atmospheric CO_2 and N_2 levels were calculated using Henry's Law at 25°C. Note that the modern ocean N and C values include both the dissolved organic and dissolved inorganic pools

concentrations have also been estimated at ~10^{-7} M (Chang 1993). Significant amounts of HCHO derived from atmospheric photochemistry (Pinto et al. 1980) would have been limited by low temperature polymerization reactions to a maximum steady-state concentration of ~10^{-4} M (Holland 1984). The possible ranges of the oceanic carbon and nitrogen species concentrations compared to the values approximated in this study are shown in Fig. 1. Other estimated constraints on the chemistry of the Archean ocean include an elevated salinity greater than present levels by a factor of ~2 (Knauth 1998) and a pH probably close to neutral (Morse and Mackenzie 1998). Given these constraints on oceanic chemistry, the formation of organic molecules in hydrothermal vent fluids, depends on the ability of input aqueous species to react and form reduced organic compounds during passage through SHSs. For SHS synthesis of amino acids to occur, kinetic pathways must exist for their formation.

Materials and Methods

Hydrothermal studies were performed using a bench-top hydrothermal vent reactor (Fig. 2) or sealed glass ampoules for longer residence time experiments. The flow-through reactor

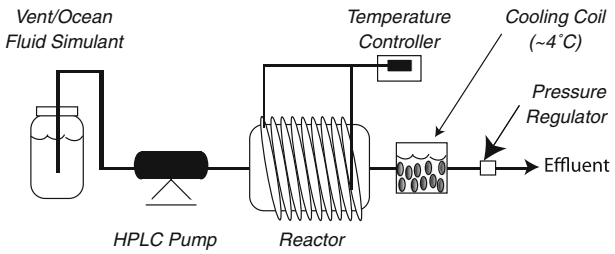


Fig. 2 Hydrothermal vent flow-through reactor schematic

consists of an Hitachi L-6200 Intelligent high-performance liquid chromatography (HPLC) pump coupled with a temperature controlled 15 mL Hastelloy C-22 (high-nickel stainless steel alloy) flow-through reactor. Temperature control is achieved using a J-Chem Scientific (Model 210) ramp-to-setpoint heater with two type-K thermocouples set deep within the hydrothermal reactor wall. Temperature control is estimated at ±5°C based on agreement of empirical high-temperature amino acid racemization rates determined using the bench-top hydrothermal reactor with reported racemization rate constants (Li and Brill 2003). The cooling stage consists of an HPLC sample loop running through a circulator bath (Haake C10-B3), and pressure control is achieved with an HPLC Upchurch Scientific PEEK P-880 backpressure regulator. The pressure is monitored by an inline pressure gauge (Omega PX305-7.5KGI) coupled with an Omega digital pressure display. The pressure gauge was calibrated against the HPLC pump pressure reading and showed consistency with these values up to ~300 bar. The post-heating cooling stage (~4°C) mimics the exit of high-temperature vent fluids into surrounding ocean waters and the backpressure regulator allows for fraction collection at ambient pressure.

Reactor composition has been shown to have significant effect on the rates of amino acid decarboxylation. For example, decarboxylation rates in quartz (Li and Brill 2003) and 316 stainless steel (Sato et al. 2004) reactors are similar, while decarboxylation in Au-Ti alloys reactors is significantly slower (Qian et al. 1993). Reactors composed of stainless steel have been documented to accelerate FTT syntheses of lipids under hydrothermal conditions (McCollom et al. 1999). The hydrothermal flow reactor exposure times utilized in this study are sufficiently short (0–60 min) to minimize reactor wall catalytic effects. Also, the formation of amino acids, by mechanisms such as the Strecker synthesis, has not been demonstrated to be catalyzed by high-nickel alloys in previous studies and should not be significant.

Short residence time exposure experiments between 10 and 60 min, corresponding to reactor flow rates between 1.5 and 0.25 mL per minute, were run using the hydrothermal reactor while experiments with residence times greater than 1 h were performed in flame-sealed glass ampoules. The flow reactor experiments were conducted as a sequence with the lowest temperature (25°C) run first and the highest temperature (~275°C) run last. Variations in the reactor flow rates provided control over solution residence times. The solution was fed into the reactor (V = 15 mL) at the appropriate flow rate (F = mL/min) for the specified residence time ($t_R = \frac{V_{REACTOR}}{F}$) set at the desired temperature. The reactor temperature was allowed to equilibrate to the setpoint temperature at the designated flow rate, one residence time equivalent of solution was permitted to pass through the reactor, and then sample fractions were collected. After 10–50 mL of solution was collected, the reactor was ramped up to the next temperature setpoint and the process was repeated.

Solutions were also sealed in pyrex ampoules and exposed to constant temperature for longer timescales in a Fisher temperature controlled drying oven (Fisher 5 cubic liter Isotemp furnace). Flow reactions were conducted at ~200 bar, typical of a seafloor hydrothermal vent environment at ~2000 m, while those in the sealed tubes are estimated to have been at ~1.5 bar. Pressure is not anticipated to be a major factor on amino acid synthetic reactions, however the datasets were evaluated independently as a precautionary measure.

Hydrothermal source material compositions included aqueous ammonium formate (NH_4HCO_2) at concentrations between 0.1 M and 0.001 M. These solutions were exposed to temperatures from 50°C to ~300°C in increments of 50°C for 20-min residence times to mimic short exposure to high-temperature hydrothermal plumes. The second set of experiments examined the effect of chemical speciation on amino acid synthesis. In natural

systems the equilibrium ratios of oxidized and reduced species would depend on the temperature, pH, residence time, H_2 fugacity, as well as the presence of minerals and metals capable of catalyzing species redox equilibration. Solutions were prepared with carbon, nitrogen, and sulfur species to mimic oxidized ($H_2CO_3^-/NO_3^-/SO_4^{2-}$), neutral ($HCOOH/NO_2^-/SO_3^{2-}$), or reduced ($HCHO/NH_3/H_2S$) hydrothermal fluid speciation (Table 2). Amino acid standards and reagents for the seawater solutions including sodium bicarbonate ($NaHCO_3$), sodium nitrate ($NaNO_3$), sodium sulfate ($NaSO_4$), formic acid (HCOOH), sodium nitrite ($NaNO_2$), sodium sulfite (Na_2SO_3), ammonium carbonate ($(NH_4)_2CO_3$), ammonium chloride (NH_4Cl), sodium sulfide (Na_2S), and ammonium formate (NH_4HCO_2) were purchased from Aldrich. HCHO (37%), NH_4OH, and NaCl were purchased from Fisher Scientific. All chemicals were ACS grade purity or higher. The solution pH values were adjusted to eight at room temperature using 1 M HCl and the ionic strength was adjusted to 0.5 M using NaCl. Solutions were prepared by dissolving the appropriate compounds in 500 mL of double distilled H_2O, and this solution was then passed through the reactor heated between 25°C and 275°C at 50°C temperature intervals. Fresh solutions were prepared each morning and exposed to high temperatures and pressures within the flow reactor or for longer residence times (>1 h) in flame-sealed pyrex tubes. Each 30 mM solution was cycled through the hydrothermal reactor as described above (25–275°C) for short residence times (10–60 min) in order to examine the short term effect of chemical speciation on amino acid synthesis and further investigations were conducted using sealed glass ampoule experiments.

All samples were desalted and derivatized after hydrothermal exposure according to published procedures (Zhao and Bada 1995) before analysis by reversed-phase HPLC (RP-HPLC). Samples were analyzed by RP-HPLC using a Phenomenex Luna-C18(2) 250×4.6 mm column, 5 uM particle size (Product #00G-4252-E0). Chromatography was carried out using a stationary phase consisting of 50 mM sodium acetate buffer (pH ~ 8.0) containing 8% methanol and a mobile phase of pure methanol (time/percent buffer: 0–5 mins/100%; 5–15 mins/63% ramp; 15–25 mins/58% ramp; 25–30 mins/40% ramp; 30–35 mins/40%; 35–45 mins/100% ramp; 45–55 mins/100%). Fluorescence detection was monitored using an excitation wavelength of 340 nm and an emission wavelength of 450 nm using a Shimadzu RF-530 fluorescence HPLC monitor.

Results and Discussion

NH_4HCO_2 is the ultimate hydrolysis product of HCN, and might be expected to accumulate to some degree in the early oceans (Stribling and Miller 1987) from rainout and hydrolysis of atmospherically synthesized (Pinto et al. 1980) or extraterrestrially delivered HCN

Table 2 Redox states for species investigated in the flow reactor

	C Species (30 mM)	N Species (30 mM)	S Species (30 mM)	pH	NaCl*
Oxidized[1]	$H_2CO_3^-$	NO_3^-	SO_4^{2-}	8	0.5 M
Neutral[2]	$HCOO^-$	NO_2^{2-}	SO_3^{2-}	8	0.5 M
Reduced[3]	HCHO	NH_3	H_2S	8	0.5 M

*All solutions normalized to 0.5 M NaCl after balancing the Na^+ reagent input

[1] Solution prepared with sodium bicarbonate, sodium nitrate, and sodium sulfate

[2] Solution prepared with formic acid, sodium nitrite, and sodium sulfite

[3] Solution prepared with formaldehyde, ammonium chloride, sodium sulfide

(Chyba and Sagan 1992). It is likely that during passage through SHSs, both formamide, which has been shown to be able to produce bioorganic compounds when heated neat or over catalysts at 100–160°C (Saladino et al. 2004), and HCN would be rapidly decomposed to NH_4HCO_2 (Miyakawa et al. 2002).

A 0.1 M NH_4HCO_2 solution produced a small suite of amino acids in low yield (~0.01% maximum) after 20-min heating times between 50°C and 292°C (Fig. 3). Methylamine and ethylamine, the decarboxylation products of glycine and alanine, respectively, were also present in the hydrothermal extracts and were most likely produced by the degradation of these amino acids after synthesis. The quantification of these degradation species is uncertain because both methylamine and ethylamine are volatile and concentrations may have been affected during sample workup. The chiral amino acids produced in the greatest yields from the heating of ammonium formate, serine and alanine, were racemic within experimental error (±5%), indicating that these are not contamination. Interestingly, the yields were maximal at temperatures between 100°C and 200°C after 20 min heating time, suggesting that these results are snapshots of the competition between synthetic reactions and decomposition reactions of product and starting material after these short exposures (Fig. 3).

The amino acids formed from 20-min exposure of ammonium formate at high-temperatures are similar to those detected in previous experiments using dry NH_4HCO_2 (Harada 1967) and from HCN polymerizations (Ferris et al. 1974), mainly glycine with smaller amounts of DL-alanine, DL-aspartic acid, and DL-serine. These reactions could be explained by a mechanism proposed for hydrogen cyanide polymerization (Fig. 3c), which involves sequential dehydration of NH_4HCO_2 to formamide ($HCONH_2$) and HCN. The dehydration of aqueous NH_4HCO_2 to HCN via $HCONH_2$ is likely concentration and temperature dependent and would thus require high concentrations of NH_4HCO_2 to form significant amounts of HCN. It is doubtful that sufficiently high concentrations of NH_4HCO_2 were available in the primitive ocean for this mechanism to have been important. HCN polymerization occurs readily from high concentrations of HCN (≥0.1 M) (Sanchez et al. 1966; Marsh and Martin 1957). If the mechanism of amino acid formation proceeds through HCN condensation, this would explain the low yields of amino acids because condensation is kinetically unfavorable due to the high activity of water, which favors hydrolysis. At higher temperatures (100–200°C) for short heating times (20 min), appreciable amounts (<0.01% yield) of amino acids were obtained.

Repetition of these experiments with 10^{-2} and 10^{-3} M NH_4HCO_2 produced no detectable amino acids (<10^{-13} moles, ~10^{-4}% yield based on nitrogen) at any temperature (50–292°C) tested, suggesting that there is a critical reactant concentration below which either reaction does not occur or synthesis is not detectable above background procedural blank amino acid levels (~10^{-13} moles). This is explicable by the fact that the synthetic reactions are likely at least 2nd order reactions, and thus highly concentration dependent, while degradation reactions are likely pseudo first-order and roughly concentration independent. It should be noted that 10^{-2} M NH_4HCO_2 already represents a very high and geochemically implausible starting concentration of fairly reactive C and N species in seawater, however despite this, no amino acids are generated in these reactions. Although these experiments were not investigated as a function of time, the low amino acid yields at high temperatures indicate that the high temperature conditions associated with axial SHSs do not favor significant amino acid synthesis and longer exposure would most likely result in degradation. These low yields combined with the fact that the primitive concentrations of NH_4HCO_2 are expected to be lower than the starting concentration of 0.1 M NH_4HCO_2 by several orders of magnitude (Miyakawa et al. 2002) suggests that condensation of ammonium formate was insignificant in primitive SHSs or the primitive oceans.

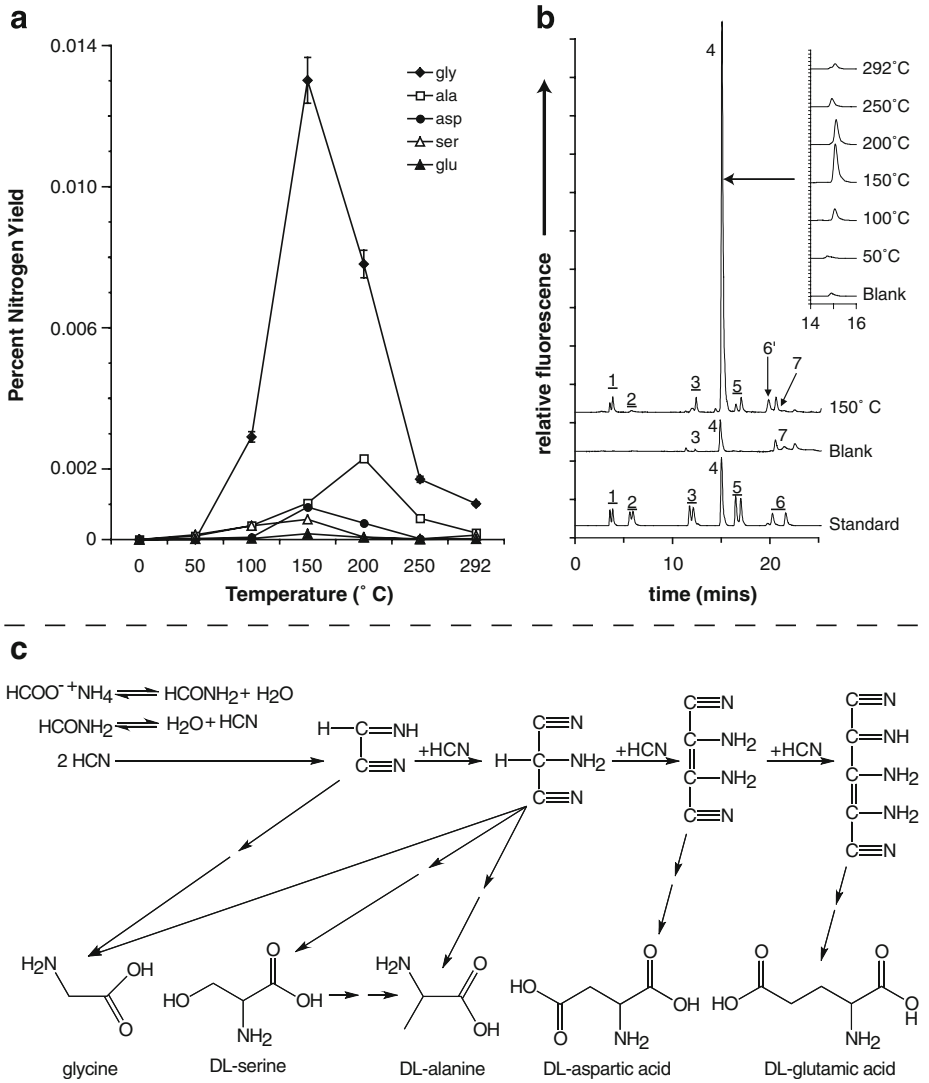


Fig. 3 **a** Yields of amino acids (Gly, Ala, Asp, Ser, Glu) produced from heating 0.1 M NH_4HCO_2 at various temperatures for 20-min at 150 atm (error bars represent ±5% measurement error), **b** 0–25 min RP-HPLC chromatograms of OPA-NAC derivatized amino acids produced from 20-min exposure at 150°C stacked against procedural blank and standard samples; inset shows the glycine peak versus temperature. 1 = D/L-aspartic acid, 2 = L/D-glutamic acid, 3 = D/L-serine, 4 = glycine, 5 = D/L-alanine, 6 = L/D-valine (6′ = L-valine coeluting peak), 7 = ammonia. Traces of β-alanine (β-ala) and γ-amino-n-butyric acid (γ-aba) were detected in some samples (the highest yields were at 100°C). All yields are based on input nitrogen (% N). **c** Possible synthetic pathway for the formation of the observed distribution of amino acids from heating experiments using NH_4HCO_2 alone as a starting material (modified from Harada 1967). The formation of amino acids from the HCN dimer through pentamer as well as the conversion of serine into alanine are likely multi-step reactions, indicated by multiple arrows

The formation of amino acids from mixtures of oxidized, reduced, or neutral species of carbon, nitrogen, and sulfur were not designed to mimic the oxidation states of the material likely to be present in vent effluent or seawater, but rather to investigate the relative reactivities of these species under SHS conditions. The starting compositions allow for an approximation of the effect of mineral buffers on the species' redox states without requiring a long equilibration time with a mineral system, and the speciation is likely to be considerably more complex (Seewald et al. 2006). The concentrations of carbon, nitrogen, and sulfur species selected (Table 2) represent relatively high estimates for the early oceans (Fig. 1). The ionic strength may be underestimated (0.5 M) and the sulfur overestimated (30 mM), but due to the uncertainties of the literature estimates for the prebiotic oceans, they were assumed to be close to the present day values.

The neutral and oxidized solutions produced no detectable amino acids ($<10^{-13}$ moles, $\sim10^{-4}$% nitrogen yield) at any temperature between 25°C and 275°C after 20 min of heating time, however the reduced species mixture produced a complex set of products, dominated by glycine, in low yield (Fig. 4a). A finer scale examination of the chromatogram reveals a suite of structurally more complicated products (Fig. 4b - Inset). The product mixture was different from that produced from NH_4HCO_2, with no detectable aspartic acid or glutamic acid. The yield of amino acids (gly, ser, ala) was greatest at temperatures between 125°C and 225°C after 20-min heating times with the maxima at 175°C (Fig. 4a).

Ammonia and HCHO react at an appreciable rate at room temperature in water to produce hexamethylenetetramine (Walker 1964), which has been reported to produce amino acids when heated (Fox and Windsor 1970). HCHO and H_2S react rapidly to produce trithiane and other low molecular weight oligomers, which have been detected in hydrothermal vent effluent (Simoneit 1995), as well as in hydrothermal laboratory experiments (Cole et al. 1994; Rushdi and Simoneit 2005). The observed amino acid products can be mechanistically explained by a series of experiments carried out by Choughuley et al. (1975) involving the addition of HCHO to glycine (Fig. 6). It should be noted that other authors (Fox and Windsor 1970; Choughuley et al. 1975; Cole et al. 1994) also found significant amounts of proline, sarcosine and betaine from similar reactions that would not have been detectable via our analytic methods, but can easily be explained within this framework, and may also be present in these reactions.

Although small but significant yields of amino acids were obtained using high concentrations of reactants, the concentration of formaldehyde in the reduced hydrothermal mixture was 30 mM, which is approximately 30 times the high-end literature estimate of 1 mM for the primitive oceans (Pinto et al. 1980). Therefore, the yields shown in these experiments represent upper limits of amino acid formation. The yield of amino acids after longer heating times was also investigated. The yields are in general dramatically lower after 185 h than after 24 h at 175°C and 125°C (Fig. 5a,b).

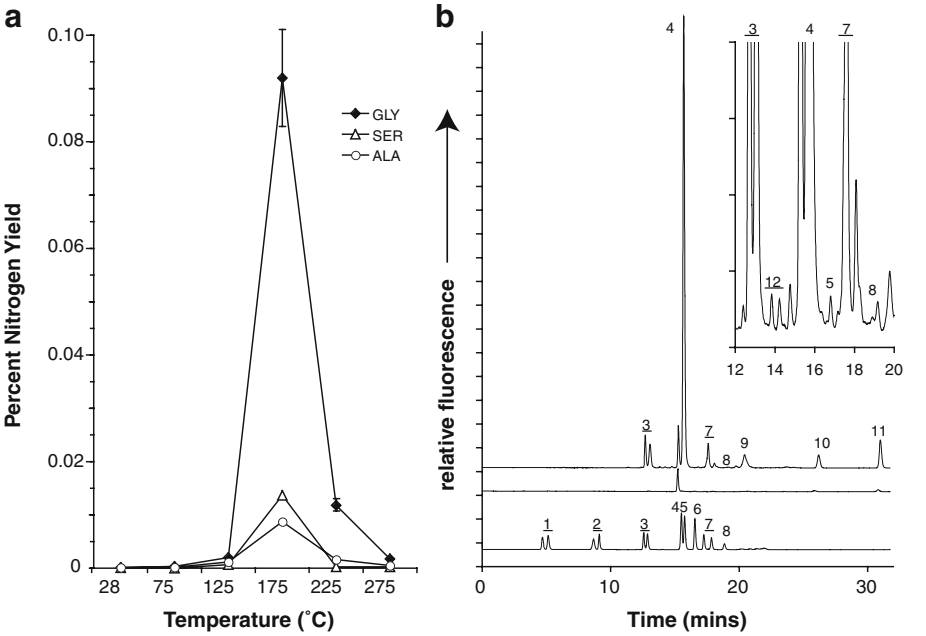
The 125°C series (Fig. 5a) showed maximal yields for glycine, serine, and alanine after ~24 h. The amino acids then degraded rapidly, presumably by deamination or decarboxylation forming methylamine, ethanolamine, and ethylamine, respectively. The amine products were detected in all of the heated fractions, but they were not quantified due to their volatility during sample workup and analysis. The 175°C temperature series showed more complicated kinetic behavior that is likely a function of the increased temperature on the amino acid formation and decomposition mechanisms occurring in the reaction. Serine showed a similar trend compared to the 125°C experiment, rapidly increasing to a maximum yield within the first 2.5 h and then degrading exponentially. Glycine and alanine are produced in maximal yield after ~75 h of heating and then yields

Fig. 4 **a** Yields of glycine, serine and alanine from 20-min hydrothermal treatment of the reduced species mixture ($HCHO/NH_3/H_2S$) as a function of temperature (bars represent ±5% measurement error), and **b** representative RP-HPLC chromatogram of OPA-NAC derivatized products from the 30 mM reduced species mixture at 175°C for 20 min (maximum yield of runs from 28–275°C) showing the formation of trace amounts of various amino acids at low and high attenuations (inset). 1 = D/L-aspartic acid, 2 = DL-glutamic acid, 3 = D/L-serine, 4 = glycine, 5 = β-alanine, 6 = γ-amino-n-butyric acid, 7 = D/L-ala (coeluting peak), 8 = α-aminoisobutyric acid (aib), 9 = ethanolamine, 10 = methylamine, 11 = ethylamine, 12 = D/L-hydroxymethylalanine. All yields based on input nitrogen (% N). **c** Possible synthetic pathway for the observed amino acids produced from heating 30 mM each Na_2S, HCHO, NH_3 and 0.5 M NaCl at pH 8 at 175°C after 30 min (modified from Choughuley et al. 1975)

diminish with time. A data point collected at 175°C after exposure for 434.5 h (~18 days) showed only blank levels of amino acids, suggesting degradation continues until the amino acids are completely decomposed (Fig. 5b). An interesting feature of the 175°C serine yield is the early maxima and subsequent decrease in yield within the first 5–10 h of reaction. The detection of racemic alanine can be explained by the dehydration of serine (Bada and Hoopes 1979) which may occur more readily at higher temperature.

Large yields of amino acids are not obtained in any case (~0.13% maximum at 125°C after ~24 h), and these drop off precipitously as a function of both temperature and time. Although few reactions were carried out past ~200 h, the data does not suggest that these reactions had obtained equilibrium with respect to amino acid synthesis and that continued heating would have resulted in further amino acid degradation. The 125°C $HCHO/NH_3/H_2S$ data show maximum yields after ~25 h, after which concentrations steadily decrease (Fig. 5a). The 175°C data show a similar trend, although maximum amino acid yields are obtained after ~75 h. Serine appears to be the precursor to alanine in these experiments and alanine would persist longer as it is more stable than serine (Bada and Hoopes 1979). Transformation of serine to alanine could account for some of the apparent persistence of amino acids at 175°C. The apparent persistence of glycine may likewise be due to degradation of initially produced serine or other amino acid products undetectable by our methods such as sarcosine. The amino acids produced may also initially become incorporated into higher molecular weight products, which then degrade into amino acids over time. The glycine or alanine measured in the first 75–100 h at 175°C in these experiments could be misinterpreted as evidence of meta-stable equilibrium were measurements not continued for longer periods of time. However, the amino acids in all of the reactions continue to decompose after 75 h.

When these experiments were repeated using 10 and 100-fold dilutions of the reduced mixture reactants (3 mM and 300 μM concentrations) with 0.5 M NaCl, the total amino acids produced were much lower at both 125°C and 175°C after 24 h of heating. For instance, the total amino acid yields from 24-h 175°C hydrothermal exposure of the 3 mM solution mixture dropped off precipitously from the original solution (~100× less than the 30 mM solution yields). These data may be explained by 2nd order or higher kinetic mechanisms whereby a drop in reactant concentration results in a much larger drop in the yield. The 125°C data show a much less drastic effect from the drop in reactant concentration implying that lower temperature syntheses (125°C) are less sensitive to concentration. This may be the effect of more complicated degradation pathways at higher temperatures (175°C). It must be pointed out that these data were collected near where the maximum yields lie for the 125°C data series (24 h exposure), so this difference may reflect sparse sampling within the highly inflected region of the observed trends (Fig. 5b). Although these lower concentration experiments were sparse, similar overall yields to those reported by Islam et al. (2001) were obtained.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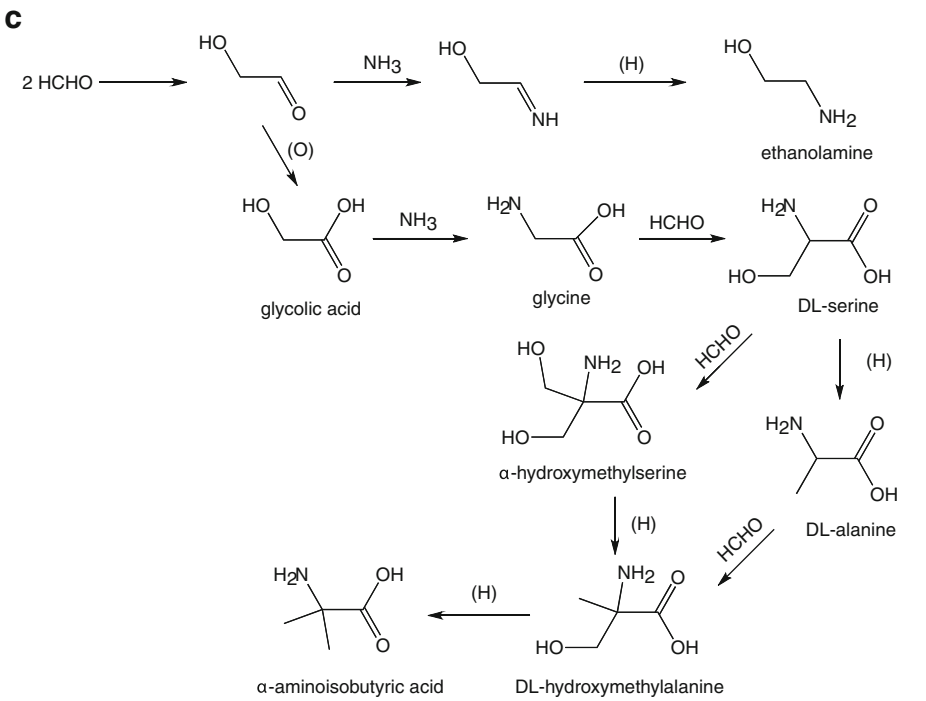
c
2 HCHO
NH3
(H)
ethanolamine
(O)
glycolic acid
NH3
glycine
HCHO
DL-serine
HCHO
α-hydroxymethylserine
(H)
DL-alanine
(H)
HCHO
(H)
α-aminoisobutyric acid
DL-hydroxymethylalanine

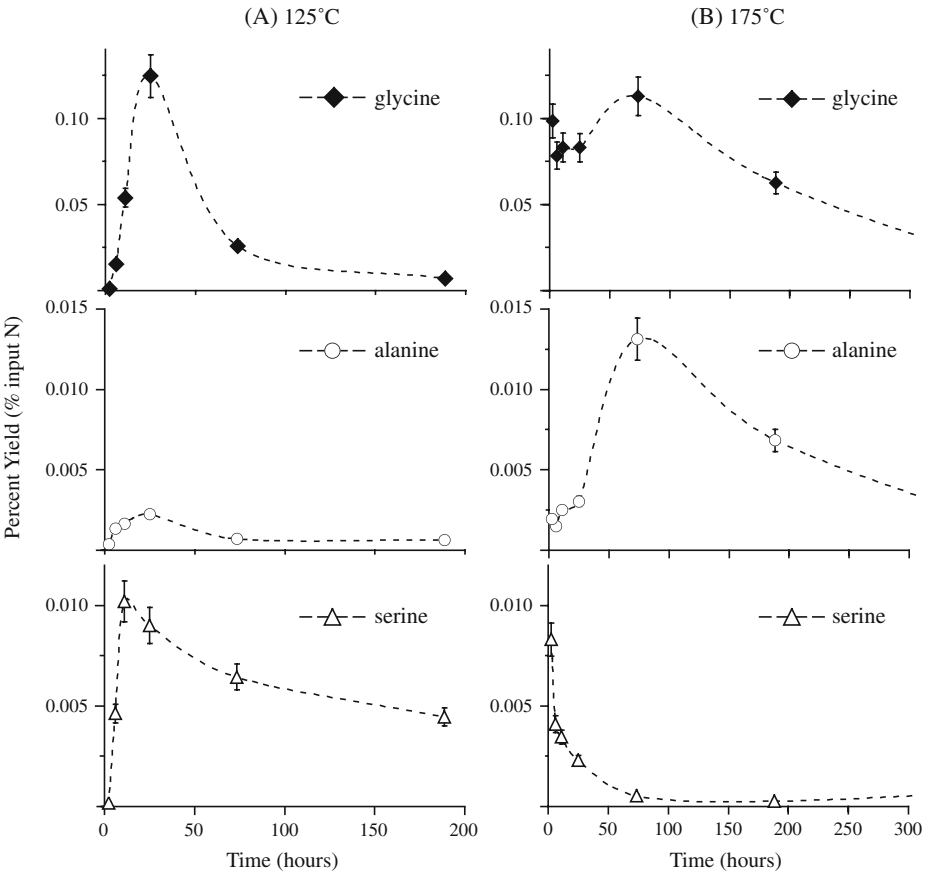

Fig. 5 Yields of glycine, serine, and alanine from the reduced species mixture (pH=8, 0.5 M NaCl, 30 mM each of Na_2S, HCHO, NH_3) as a function of heating time at **a** 125°C for up to ~190 h and **b** 175°C up to ~300 h. The 175°C temperature series was monitored to 434.5 h of heating at which point no amino acids were detected ($<10^{-4}$% nitrogen yield). Yields are reported based on input nitrogen (%N). The curve fits are only intended as guides for the eye

The effect of the presence of O_2, NaCl and sulfide on the reduced reaction mix were also investigated. Experiments conducted after degassing to remove O_2 showed slightly higher overall amino acid yields, relative to those which were not degassed, after 100 h of exposure at 175°C (~20% higher yields, ~0.1% overall yield). Excluding Na_2S from the reaction mixture had little effect on the amino acid yields. Amino acid yields from experiments run in the absence of NaCl with and without Na_2S were <10% of the 30 mM complete mixture yields, suggesting that NaCl may have an important effect on synthesis. This may be due to pH effects, as the buffering capacity of the system at high temperatures is probably rather poor, or may be due to ionic strength effects in high temperature synthesis.

The effects of pressure, H_2 fugacity, and pH on amino acid synthesis and stability were not investigated in these experiments as the aim of these studies was to evaluate the formation of amino acids as a function of the concentration and oxidation state of various seawater species. It is questionable whether adequate concentrations of N and C compounds have ever been present in SHS environments to allow for significant synthesis of amino

acids as our experiments showed a very large concentration dependence on amino acid formation. Increasing H_2 fugacity may give results more consistent with the reduced species mixture, which showed the highest yields (Fig. 5), by allowing greater equilibrium concentrations of reduced organic compounds such as CH_4 and CO, while lowering the rates of degradation. Increased H_2 fugacity, produced from high-temperature interactions of basalt with seawater, has been suggested to stabilize amino acids (Shock 1992a; Qian et al. 1993; Kohara et al. 1997; Islam et al. 2001) and increase amino acid synthesis under SHS conditions (Hennet et al. 1992; Andersson and Holm 2000). However, the effect of H_2 fugacity on amino acid stability has been studied and the effect is relatively slight, with half-lives being longer by at most a factor of 2 (Islam et al. 2001).

Previous estimates of equilibrium concentrations of amino acids in SHS fluids have depended on computational models rather than laboratory data (Shock 1990, 1992b; Schulte and Shock 1995; Amend and Shock 1998; Shock and Schulte 1998), however most of these calculations have not yet been validated experimentally. The major weaknesses of such models, besides uncertainties associated with the thermodynamic constants, are that they neglect the kinetic aspects of amino acid synthesis. Both the 125°C and 175°C temperature series show amino acid yields consistent with a simplified generalized reaction scheme $A \rightleftharpoons B \rightleftharpoons C$ where the amino acids are represented by "B", the precursors as "A", and the amino acid degradation products as "C". A schematic for glycine is presented (Fig. 6) where simultaneous degradation of glycolic acid among other reactions would compete with glycine formation. The equilibrium formation of the even simplest amino acid is thus a complicated reaction to model.

The overall ratios of the rates of k_1/k_{-1} and k_2/k_{-2} then control the observed yield of amino acids at a given time, although there could obviously be, and likely are, many other important side reactions such as decomposition of the starting material and reactions of the various intermediates with each other. If it is assumed that the primary pathway for glycine formation is the amination of glycolic acid (Fig. 6), then equilibrium concentration of glycine can be roughly estimated. The amination equilibrium of glycolic acid and glycine can be approximated by the reaction of fumaric acid and ammonia to form aspartic acid (NH_3 + HOOC-$(CH)_2$-COOH → HOOC-CH2-CHNH2-COOH). The equilibrium constant for this

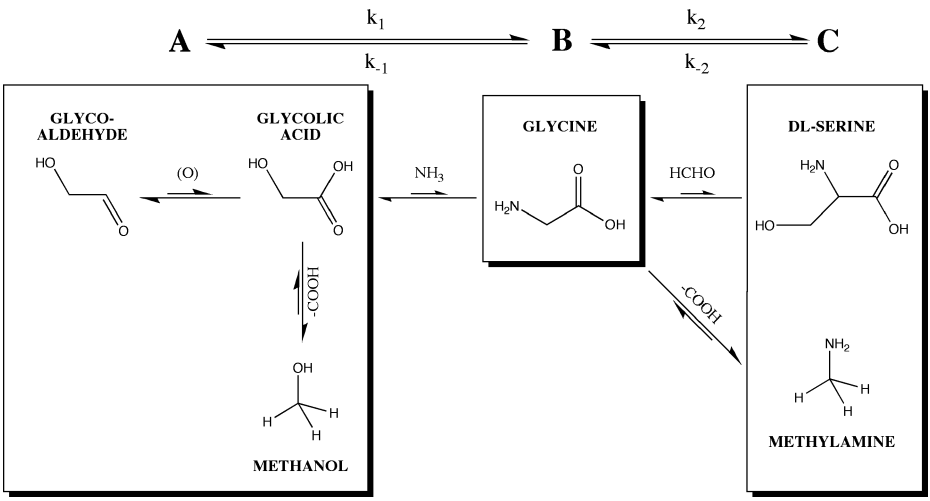

Fig. 6 Simplified kinetic reaction scheme for glycine formation

reaction is estimated as between 0.011 and 0.0024 at 25°C (Borsook and Huffman 1933), and was discussed in more detail as a function of temperature by Bada and Miller (1968b). Assuming 1 mM concentrations of ammonia and 10 mM concentrations of fumaric acid, the variation with temperature of equilibrium concentrations of DL-aspartate can be estimated as ~$2.0–2.5\times10^{-7}$ between 125°C and 175°C, if there are no other loss channels for aspartate or fumarate. The free energy of hydrolytic deamination of aspartic acid to fumaric acid (Equation 1, k_{-1}) is ~4.75 kcal/mole (Bada and Miller 1968b), and the free energy for the amination of glycolic acid to glycine is reportedly similar (Peltzer et al. 1984). However, aspartate is one of the few amino acids that easily undergoes reversible amination (Sohn and Ho 1995) and therefore the equilibrium between glycine and glycolic acid is most likely significantly less than 2×10^{-7}. Experimental evidence suggests that the competing degradation pathways for both methylamine and glycolic acid tend towards the formation of simpler species such as NH_3, CO_2 and H_2 (Bada et al. 1995). Therefore, if catalysts or sufficient time exists for equilibration to occur, the reactions of reduced C and N species should produce concentrations of amino acids which are likely lower than those estimated by the simple amination reactions estimated above. There are obviously many other reactions that might be considered in establishing these equilibria, however each reaction generally favors decomposition at high temperature over even short geological time scales. Shock (1992a) has calculated the activity of glycine in a 10 bar CO_2, 1 bar N_2, H_2 rich, high temperature vent environment as 10^{-9} to 10^{-19}. As the concentrations of amino acids in all of our experiments appear to be decreasing with time, it may well be that they are converging on these equilibria. Such equilibrium concentrations would be below our detection limit if reasonable starting concentrations of C and N species were used.

Conclusion

We have herein modeled possible high temperature amino acid synthesis processes using extremely favorable conditions and verified that a limited set of amino acids are generated in low yields from concentrated reactive compounds at high temperatures provided the solutions are not heated very long. High concentrations of HCHO and NH_3 might produce significant amounts of amino acids at lower temperatures in the open oceans given sufficient time, however the concentrations of starting materials in the primitive oceans were likely too low to have been useful for this type of synthesis (Bada and Miller 1968a; Holland 1984; Miyakawa et al. 2002). Even if the prebiotic oceans contained significant amounts of C and N species, the increase in synthetic reaction rates at high temperatures would compete with concomitant degradation reactions and limit the accumulation of organic compounds. Previous measurements of the decomposition rates of amino acids (Bernhardt et al. 1984; White 1984; Miller and Bada 1988; Bada et al. 1995; Ito et al. 2006) appear to show irreversible pseudo-first order kinetics because the equilibria for these reactions are extremely low. Using a high-end equilibrium estimate of 10^{-9} M (Shock 1992a), this would become the limiting concentration of amino acids in the ocean due to cycling through SHSs. High temperatures in hydrothermal vent systems would then be limiting due to destruction.

According to these results, lower temperature submarine hydrothermal systems (~100°C) might be more important for hydrothermal organic synthesis. Mixing of hydrothermal plume fluids with low temperature seawater or fluids associated with off axis SHS environments such as those emanating from lower temperature seeps, within “beehive” hydrothermal vent systems (Fouquet et al. 1993), or within older oceanic crust such as at the Lost City hydrothermal field (Kelly et al. 2001) offer wide ranges of lower temperature regimes which

might be more hospitable for organic synthesis. Traces of what appear to be abiogenic methane and straight-chain hydrocarbons have been detected in a variety of ultramafic-hosted hydrothermal systems (Charlou et al. 2000, 2002; Proskurowski et al. 2008), however the detection of abiogenic amino acids in hydrothermal vent fluids remains elusive. This is either because abiotic amino acid synthesis does not occur in these environments or because it occurs at levels below analytical sensitivity or below the threshold necessary to distinguish them from background biological contamination (Takano et al. 2003). This study suggests that amino acids are not detected because the reduced starting compounds and intermediates necessary for their formation are degraded rapidly at high temperatures. Short or intermittent exposure at elevated temperatures could solve this problem but even in these lower-temperature regimes, extremely low reactant concentrations likely also limit synthesis.

This study neglects the possible influence of minerals on these syntheses. It is possible that FTT synthesis might produce similar products if NH_3 and CH_4 or CO were abundant species in vent environments and catalysts were present which could convert them to organic compounds (Yanagawa and Kobayashi 1992). For instance, mineral surface catalysis via FTT synthetic pathways has been suggested to account for the formation of methane and hydrocarbons detected in vent effluent (Foustoukos and Seyfried 2004), and experiments suggest the catalytic production of small amounts of methane (Horita and Berndt 1999) and hydrocarbons (Berndt et al. 1996) from aqueous CO_2 by similar mechanisms. The formation of amino acids, however, has not been demonstrated under reasonable submarine hydrothermal system environmental conditions, and we suggest here that the initial concentrations of reduced precursors are a fundamental barrier for such syntheses. It is unlikely that mineral adsorption would offer significant protection for amino acids, as they would still experience considerable aqueous phase degradation during their long residences in SHSs. The adsorption of amino acids to mineral surfaces generally decreases with increasing temperature (Ito et al. 2006), and model results predict that the reducing power of iron-sulfur mineral assemblages decreases with increasing temperature (Schoonen and Xu 2001).

Although kinetic pathways for amino acid synthesis from reactive compounds exist, extrapolation of these experimental data to geochemically relevant starting material concentrations and long exposure times within SHSs, suggests it is unlikely that high temperature environments were significant for the synthesis of amino acids, although they may have contributed small amounts of less labile organic material such as acetate to the general pool of organics in the primitive environment.

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