1 **Dissolved amino acids in oceanic basaltic basement fluids**

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23 **Abstract**

24 The oceanic basaltic basement contains the largest aquifer on Earth and potentially plays
25 an important role in the global carbon cycle as a net sink for dissolved organic carbon
26 (DOC). However, few details of the organic matter cycling in the subsurface are known
27 because great water depths and thick sediments typically hinder direct access to this
28 environment. In an effort to examine the role of water-rock-microorganism interaction on
29 organic matter cycling in the oceanic basaltic crust, basement fluid samples collected
30 from three borehole observatories installed on the eastern flank of the Juan de Fuca Ridge
31 were analyzed for dissolved amino acids. Our data show that dissolved free amino acids
32 (1-13 nM) and dissolved hydrolyzable amino acids (43-89 nM) are present in the
33 basement. The amino acid concentrations in the ridge-flank basement fluids are at the low
34 end of all submarine hydrothermal fluids reported in the literature and are similar to those
35 in deep seawater. Amino acids in recharging deep seawater, *in situ* amino acid production,
36 and diffusional input from overlying sediments are potential sources of amino acids in the
37 basement fluids. Thermodynamic modeling shows that amino acid synthesis in the
38 basement can be sustained by energy supplied from inorganic substrates via
39 chemolithotrophic metabolisms. Furthermore, an analysis of amino acid concentrations
40 and compositions in basement fluids support the notion that heterotrophic activity is
41 ongoing. Similarly, the enrichment of acidic amino acids and depletion of hydrophobic
42 ones relative to sedimentary particulate organic matter suggests that surface sorption and
43 desorption also alters amino acids in the basaltic basement. In summary, although the
44 oceanic basement aquifer is a net sink for deep seawater DOC, similar amino acid
45 concentrations in basement aquifer and deep seawater suggest that DOC is preferentially

46 removed in the basement over dissolved amino acids. Our data also suggest that organic
47 carbon cycling occurs in the oceanic basaltic basement, where an active subsurface
48 biosphere is likely responsible for amino acid synthesis and degradation.

1. Introduction

Containing a volume of hydrothermal fluids equivalent to ~ 2% of the global ocean (Johnson and Pruis, 2003), the upper 40-500 m of basaltic ocean basement is the largest aquifer on Earth (Fisher, 2005). The basement fluids are not stationary: the flux of hydrothermal water through ridge flanks—crustal age older than 1 million years— is estimated to be equivalent to 5-60% of the global riverine input of water into the ocean (Mottl, 2003; Wheat et al., 2003). The hydrothermal flux is sufficiently large that the entire ocean is recycled through the basaltic basement once every 100,000 to 500,000 years (Fisher, 2005). Thus, the circulation of deep ocean water through submarine hydrothermal systems plays an important role in global biogeochemical cycles, including the global carbon cycle. This is due to the fact that there is nearly as much fixed carbon as dissolved organic carbon (DOC) in the deep ocean (>1000m; 662 Pg C; Hansell et al., 2009) as there is CO₂ in the atmosphere (750 Pg C; Siegenthaler and Sarmiento, 1993), and that seawater DOC is removed during its flow path in the basement (Lang et al., 2006; Lin et al., 2012).

Biological activity in the subsurface has been suggested to play a role in DOC removal (Lang et al., 2006; Lin et al., 2012). There are several lines of evidence, including the texture, chemical, and isotopic composition of both basaltic glass and rocks, suggesting the existence of biological activity within the ridge-flank basement (Giovannoni et al., 1996; Fisk et al., 1998; Furnes et al., 2006; Rouxel et al., 2008; Wheat et al., 2010; Alford et al., 2011; Alt and Shanks, 2011; Ono et al., 2012). Fluids sampled from the ridge-flank aquifer have yielded ribosomal RNA gene sequences related to chemolithoautotrophs and heterotrophs, and functional genes of sulfate reducers,

indicating the presence of a diverse bacterial and archaeal community (Cowen et al., 2003; Huber et al., 2006; Jungbluth et al., 2013; Robador et al., 2015). However, these genomic data do not necessarily reveal what, if any, heterotrophic activity is ongoing in this environment. In addition, although few studies have investigated the energetic potential for chemolithoautotrophic metabolisms in the oceanic basaltic aquifer (Bach and Edwards, 2003; Lin et al., 2012; Boettger et al., 2013), there is no investigation focused specifically on understanding the energetic cost of synthesizing or the yield from degrading key biomolecules such as amino acids.

As the building blocks of all proteins and potential sources of carbon, nitrogen, and energy, amino acids (AAs) are one of the most bio-utilizable (or labile) organic substrates in aqueous environments (Crawford et al., 1974; Wheeler and Kirchman, 1986; Fuhrman, 1987; Keil and Kirchman, 1991b; Middelboe et al., 1995; Ouverney and Fuhrman, 2000). Although many microorganisms isolated from hydrothermal environments have been shown to actively take up AAs (Jannasch et al., 1988; Pledger and Baross, 1991; Hoaki et al., 1993; Hoaki et al., 1994; Dirmeier et al., 1998), the distribution and abundance of AAs within hydrothermal ecosystems have only been documented in a few studies (Haberstroh and Karl, 1989; Takano et al., 2003; Svensson et al., 2004; Klevenz et al., 2010; Lang et al., 2013). In addition, due to preferential removal and transformation of various AAs during microbial organic matter degradation, the compositions of protein-forming and non-protein forming AAs can be used to examine the diagenetic status of organic matter to infer the occurrence of heterotrophic activities in an environment (Hecky et al., 1973; Cowie and Hedges, 1992; Dauwe et al., 1999; Heeschen et al., 2003; Aluwihare and Meador, 2008). This status, however, has not

yet been documented for any submarine hydrothermal environment, largely due to the difficulty of obtaining fluid samples from the deep seafloor.

Recent advances in the installation of Circulation Obviation Retrofit Kit observatories (CORKs) into Integrated Ocean Drilling Program (IODP) boreholes have provided unprecedented access to the deep basaltic basement. In an effort to study water-rock-microorganism interactions on organic matter cycling, we use the concentration and composition data of dissolved AAs in ridge-flank basement fluids to infer processes involving AA cycling in the basaltic basement. To provide context, the AA data are compared to the concentrations of AAs in other submarine hydrothermal systems and deep seawater. Thermodynamic calculations are presented to evaluate the energetics of AA syntheses in the basement. Several established AA-based degradation indices are also calculated to examine the diagenetic status of organic matter in basement fluids.

2. Methods and materials

2.1 Sampling methods

Pristine basement fluid samples were collected from CORKs installed at IODP Holes U1301A, U1362A, and U1362B located on the eastern flank of Juan de Fuca Ridge off the western coast of North America (Figure 1). Penetrating through ~ 250 m of sediment into the ~ 3.5 million year old basement, the three CORKs are equipped with fluid delivery lines made of polytetrafluoroethylene (1362A and 1362B) or stainless steel (1301A; Shipboard Scientific Party, 2004; Expedition 327 Scientists, 2010). Based on geochemical composition, basement fluid samples collected using a clean pumping system via fluid delivery lines are superior to those flowing through the reactive, rusty

iron casing found in most CORKs (Cowen et al., 2012; Lin et al., 2012). The three sites are within a radius of 1 km and have fluid intake depths at 30 m (1301A), 40 m (1362B), and 200 m (1362A) below the sub-basement, allowing for the investigation of spatial differences in labile organic carbon cycling. Sampling methods for basement fluid used in this study are described in Cowen et al. (2012) and Lin et al. (2012). Briefly, a mobile pumping system (MPS), transported and powered by the remotely operated vehicle (ROV) *Jason* or the human-occupied vehicle (HOV) *Alvin* was used to pump the fluid up through CORKs' fluid delivery lines and into 60 L Tedlar (polyvinyl fluoride) bag samplers (LVBS) in 2009 and 2010 from CORK 1301A, and 15 L foil-lined, gamma irradiated polyethylene inner-liner bag samplers (MVBS) from CORKs 1301A, 1362A and 1362B in 2011. Aliquots of basement fluids were subsampled into acid-cleaned, 20 mL combusted (550°C, five hours) glass scintillation vials once the LVBS or MVBS were shipboard. Subsampling was conducted inside a laminar flow hood with a high-efficiency particulate air filter. The fluid samples were then stored frozen at - 20°C until analysis. Sampling blanks, including LVBS and MVBS sampling and shipboard subsampling made by processing deionized water, do not show significant AA contamination.

2.2 Amino acids analysis

2.2.1 Hydrolysis

Fluid samples were hydrolyzed to break down dissolved combined AAs (DCAAs), such as peptides and small proteins, into free AAs for quantification. In order to facilitate comparison of AA concentration to those reported in the literature, the concentrations of

total dissolved hydrolysable amino acids (DHAAs) are taken to be the sum of dissolved free amino acids (DFAAs, measured without hydrolysis) and DCAAs. Vapor phase hydrolysis was performed using a modified procedure described by Keil and Kirchman (1991b) with a vacuum-heating hydrolysis system (PicoTagTM, Waters). A 100 μ L fluid sample was pipetted into a muffled 1 mL glass insert and placed into a 40 mL hydrolysis reaction vessel where samples were dried under vacuum ($< 80 \times 10^{-6}$ bar). Then, 2.1 mL of 7N HCl, 10% v/v trifluoroacetic acid with 0.1 % v/v phenol was added to the reaction vessel. Samples were hydrolyzed in the vapor phase at 156°C for 23 minutes under an anoxic nitrogen atmosphere. After hydrolysis, samples were dried under vacuum to remove the acid, and the hydrolysate was dissolved in 100 μ L deionized water for subsequent free AA analysis by HPLC.

2.2.2 Amino acid characterization and quantification

Characterization and quantification of AA concentrations were carried out in an automated High Performance Liquid Chromatography (HPLC) system (Alliance Separation Module 2690, Waters). The stationary phase was a 2.1 mm $\times$ 150 mm Hypersil Gold column packed with 3 μ m C-18 spheres (Thermo Scientific). The pH was first adjusted by adding a one-to-ten volume ratio of borate buffer (0.4 M, pH 10.5) into the sample (Grasshoff et al., 1999; Svensson et al., 2004). Then, an o-phthalaldehyde derivatization reagent (100 mg of o-phthalaldehyde in 0.5 mL methanol and 0.05 mL of 2-mercaptoethanol) was added into pH-adjusted samples at a 1:100 ratio (Grasshoff et al., 1999) by the auto-sampler and allowed to react with the sample for exactly 10 minutes; 30 μ L of derivatized sample were then injected into the separation column.

The mobile phases of the separation were 20 mM sodium acetate trihydrate with 0.3% v/v tetrahydrofuran (pH adjusted to 7.1 using 2% v/v acetic acid) and 100% methanol. The gradient started with 15% methanol and increased to 18% in the first 4 minutes. Then methanol increased to 22 % at 6.7 minutes, 38 % at 25 minutes, 50 % at 40 minutes and 100% at 47 minutes. The gradient was followed by 7 minutes of 100% methanol for column cleanup. The gradient was returned to initial conditions (15% methanol) for 6 minutes to equilibrate the column for the next analysis. This gradient system can separate 15 protein-forming AAs (aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine, see [Figure 2](#)) and two non-protein-forming AAs (γ -aminobutyric acid and β -alanine). The o-phthalaldehyde derivatized AAs were detected by a fluorescence detector (RF-20Axs, Shimadzu) with excitation and emission wavelengths set at 340 nm and at 420 nm, respectively. Please refer to the Appendix for details on detection limits ([Table A1](#)), blanks ([Table A2 and A3](#)) and peak assignments ([Table A4](#)).

2.2.3 Calibration and limits of detection

Calibrations of AAs were made with mixed Amino Acid Standard-H, γ -aminobutyric acid and β -alanine standards (Thermo Scientific Pierce). Since tryptophan degraded (e.g. [Coffin, 1989](#)), asparagine turned into aspartic acid, and glutamine turned into glutamic acid during acid hydrolysis (e.g. [Keil and Kirchman, 1991b](#)), these three AAs were not calibrated and reported. Limits of detection (signal to noise ratio =3) were smaller than 3-30 fmol, which is equivalent to 0.1-1 nM at the 30 μ L injection volume for

individual AAs. Reagent blanks were estimated by analyzing deionized water every day and were 0.1-3 nM of individual AAs. The reagent blank was subtracted from the DFAA measured on the same day since o-phthalaldehyde reagent was made daily. Hydrolysis blanks monitored for each batch of hydrolysis ranged from 16-42 nM total AAs and were subtracted from the samples hydrolyzed at the same time. Lysine was much less sensitive than all other AAs using the chosen column and derivatization reagent and had a limit of detection of ~ 10 nM.

3. Calculations

3.1 Thermodynamic calculations

Gibbs energies of reaction (ΔG_r) are calculated as

$$\Delta G_r = \Delta G_r^0 + RT \ln Q_r, \quad (1)$$

where ΔG_r^0 stands for the standard molal Gibbs energy of each reaction at the temperature and pressure of interest, R and T refer to the gas constant and temperature in Kelvin, respectively, and Q_r represents the reaction quotient, which is calculated using

$$Q_r = \prod (a_i^{v_{i,r}}), \quad (2)$$

where a_i denotes the activity of chemical species i , and $v_{i,r}$ represents its stoichiometric coefficient. Activities were calculated using the concentrations of the species and their individual ion activity coefficients, which were in turn calculated using an extended version of the Debye-Hückel equation (Helgeson, 1969) and Geochemist's Workbench at the *in situ* temperature. Values of ΔG_r^0 at the *in situ* temperature (65°C) and pressure (270 bar) were computed using the revised HKF equations of state (Helgeson et al., 1981;

204 Tanger and Helgeson, 1988; Shock et al., 1992), the SUPCRT 92 software package
 205 (Johnson et al., 1992), and thermodynamic data taken from the literature (Shock and
 206 Helgeson, 1988; Shock et al., 1989; Amend and Helgeson, 1997; Schulte et al., 2001;
 207 Dick et al., 2006; LaRowe and Dick, 2012). The reactions describing the synthesis of
 208 AAs and the respective equilibrium constants, K , used for thermodynamic calculation are
 209 listed in Table 1. Values of K are related to ΔG_r^0 through

$$210 \quad \Delta G_r^0 = -2.3RT \log K \quad (3)$$

211 The physical and geochemical data used to calculate values of Q_r are shown in Table 2.
 212 The average concentrations of detected DFAs, major ions, ammonium, bicarbonate and
 213 sulfide reported in Table 3 were used in these calculations. For the undetected DFA,
 214 concentrations were set to their quantification limits. Activities of H_2 in ridge-flank
 215 basement fluids were computed from concentrations (0.05-1.8 μM) reported previously
 216 (Lin et al., 2012 and Lin et al., 2014).

217 **3.2 Degradation Index**

218 To infer the degree to which organic matter has degraded, the proportions of AAs
 219 in a given sample are compared to the abundance of AAs in reference organic materials.
 220 The resulting Degradation Index (DI) is calculated using (Dauwe et al., 1999):

$$221 \quad DI = \sum_i \left[\frac{\text{var}_i - \text{AVGvar}_i}{\text{STDvar}_i} \right] \times \text{fac coef}_i \quad (4)$$

222 where var_i represents the mole percentage of the i th AA, AVGvar_i and STDvar_i refer to
 223 the mean and standard deviation of the data set, respectively, and fac coef_i corresponds to

the factor coefficient for the i th AA. Factor coefficients (Table A5) used here are the unrotated loadings from principle component analysis based on a dataset covering the possible range of degradation states in marine dissolved organic matter (DOM, Kawasaki and Benner, 2006; Davis and Benner, 2007; Kaiser and Benner, 2008; Kaiser and Benner, 2009). DOM-based factor coefficients are used instead of the original particulate-organic-matter-based factor coefficients reported in Dauwe et al. (1999) because AA compositions in DOM are not comparable to AA compositions in particulate organic matter (Kaiser and Benner, 2009). In this study, fresh marine-algal-derived DOM (Amon et al., 2001) and North Atlantic surface and deep seawater DOM (Kaiser and Benner, 2009) are used as references for diagenetically unaltered, slightly altered and extensively altered DOM, respectively, for comparison with basement fluid DOM in Eq. (4). Note that the DI values of a sample can only be compared with those included in the same data set used for the calculation; in other words, we cannot compare the DI values calculated here with those reported in literature such as Dauwe et al. (1999).

4. Results and discussion

4.1 Concentrations of amino acids in basement fluids

The concentrations of DFAA and DHAA represent the extent to which amino acid production exceeds or lags behind amino acid removal. In order to infer mechanisms controlling AAs, concentrations of DFAA and DHAA in basement fluids are compared with those in fluids from various hydrothermal ecosystems and deep seawater reported in the literature (Table 5). Except for undetectable DFAA ($< 0.001 \mu\text{M}$) in the Guaymas Basin vent fluid, concentrations of DFAA (0.001-0.014 μM) and DHAA (0.043-0.089 μM , Table 4) in the basement fluid samples were at the low end of those in any other

non-ridge-flank submarine hydrothermal vent fluids (Table 5, DFAA 0.035-78 μM ; DHAA 0.067-35 μM ; Svensson et al., 2004; Klevenz et al., 2010; Lang et al., 2013) and were much lower than those in hydrothermally-influenced sediment porewater samples (DFAA 0.27-446 μM ; DHAA 0.8-338 μM ; Haberstroh and Karl, 1989; Hoaki et al., 1995). The low DHAA concentrations in the basement fluids are, however, within the range of those in the deep seawater (0.05-0.18 μM , Lee and Bada, 1975; Druffel et al., 1992; Kaiser and Benner, 2009).

The undetectable DFAA in the Guaymas Basin fluids have been explained as the result of the instability of amino acids at extremely high temperatures and pressure (Haberstroh and Karl, 1989). Such an interpretation is confirmed by laboratory experiments that show complete decomposition of amino acids at 250°C after 240 hours (Ito et al., 2006). Although the same amino acid decomposition experiment did not cover the temperature of our study sites (65°C), much less decomposition was observed in the same study at 100°C than at higher temperatures (Ito et al., 2006). We infer that complete thermal decomposition of amino acids is minor at the modest temperatures (65°C) within the ridge-flank basement environment; thus, the presence of DFAA is not surprising. However, higher concentrations of DFAA in the mid-Atlantic high temperature vent fluid samples (Table 5) than those in the basement fluids are unexpected as heat degradation at 300°C should have destroyed the DFAA as suggested by Ito et al. (2006); no explanation of this issue has been provided by Klevenz et al. (2010) but AA contamination during sampling is plausible.

The high concentrations of DFAA and DHAA in fluids from other non-ridge-flank hydrothermal vent systems and hydrothermal influenced sediment listed in Table 5

are attributed to large supplies of particulate organic matter—from biological production or from organic-rich sediments—coupled to intensified acid hydrolysis due to low pH (down to pH=2) and thermal decomposition (up to 104°C), which often leads to DFAA/DHAA ratios as high as 0.33-0.37 (Horiuchi et al., 2004; Svensson et al., 2004; Lang et al., 2013). However, the oceanic basement fluids considered in this study were at near-neutral pH (~ 7.4) with a moderate temperature (65°C) and very low particulate organic carbon contents (0.01-2 µmol/L), which do not conspire to produce amino acids. In addition, chemolithoautotrophy is more apparent in the comparing non-ridge-hydrothermal systems (Kelley et al., 2005; Lang et al., 2013) than the ridge-flank system. Some shallower vent sites and hydrothermal influenced sediment are located where photoautotrophy is also a potential source for AAs (Hoaki et al., 1995; Wenzhöfer et al., 2000).

The low AA concentrations in basement fluids, on the other hand, are similar to those in seawater. The DFAA/DCAA is only 0.02-0.18 in ridge flank basement fluids, which is similar to those observed in near-shore and open ocean seawater (Lee and Bada, 1975; Coffin, 1989). In sum, unlike non-ridge-flank hydrothermal systems, heat degradation and acid hydrolysis of particulate organic matter are likely not major controls on the production of AAs in the ridge-flank subsurface hydrothermal systems. It is more likely that the prevailing concentration of AAs in the basement are due to a combination of seawater recharge, *in situ* production and transportation from overlying sediment, heterotrophic consumption, and surface adsorption.

4.2 Amino acid cycling in the basement fluids—potential sources and sinks

4.2.1 *Input of amino acids from recharging seawater*

Basement fluids originate from deep seawater recharging through permeable unsedimented or thinly sedimented mid-ocean ridge or seamounts (Fisher et al., 2003; Hutnak et al., 2006; Fisher and Wheat, 2010; Wheat et al., 2013). In the deep Pacific Ocean (> 1000 m), dissolved protein-like DOM, i.e., DHAA, is significantly older, by three to four thousand years, than the amino acids in particulate organic matter and are thus deduced to be less reactive to bacteria than younger particulate amino acids (Loh et al., 2004). If such protein-like material remains unreactive in the basement, its presence can explain the similarly low DHAA concentrations in the basement fluids and deep seawater (Table 5). However, Loh et al. (2004) suggest that although protein-like materials are significantly aged, they comprise the younger fractions of the DOM, and thus are part of the more reactive components of deep seawater DOM. Because different DHAA compositions are found in samples collected from different sites and times, basement fluid DHAA—including those from recharging deep seawater—are deduced to be reactive and/or derived from additional sources.

4.2.2 *In situ* production in the basement

Biological activity has been inferred from traces of active chemolithoautotrophic sulfate reduction within the ridge-flank basement environment, and it is supported by the low $\delta^{34}\text{S}$ values of pyrite from basaltic rock collected from site 1301 cores (Ono et al., 2012; Lever et al., 2013). Sulfate metabolism at site 1301A has also been inferred from sulfate reduction rate measurements and phylogenetic analysis (Robador et al., 2015). The carbon and hydrogen isotopic compositions of methane also suggest that microbial

methanogenesis is responsible for the methane observed in CORKs 1362A and 1362B basement fluids while isotopically heavy methane infers biological methane oxidation in CORK 1301A (Lin et al., 2014). Based on carbon isotopic compositions, the high molecular weight DOC in basement fluids is also thought to be synthesized by chemosynthetic microbial communities in these fluids (McCarthy et al., 2010). Therefore, it is very likely that *in situ* chemolithoautotrophic production of amino acids (free or combined) also occurs. The basement fluids, despite their presumed low energy potential (Boettger et al., 2013), are conducive to a wide variety of chemolithoautotrophic metabolisms. For example, although energy is required for synthesizing AA at CORK 1301A ($\Delta G_r = 4$ to 17 kJ/mol e^- , Figure 3), energy can be released from hydrogenotrophic methanogenesis ($\Delta G_r = -5$ kJ/mol e^-), hydrogenotrophic sulfate reduction ($\Delta G_r = -12$ kJ/mol e^-) or hydrogenotrophic iron reduction ($\Delta G_r = -48$ kJ/mol e^-) at CORK 1301A (Boettger et al., 2013). Due to much higher concentrations of sulfate (18.8 mM), bicarbonate (359 μ M), and hydrogen (0.05-1.1 μ M) relative to AAs (few nM), inorganic substrates in basement fluids can provide sufficient energy for microorganisms such as methanogens and sulfate reducers to make all of the amino acids observed in the basement environment.

Thermodynamic calculations also demonstrate that putative microbes living near the sites with higher hydrogen concentrations (such as CORK 1301A in 2010) have an anabolic advantage over microbes living in other sites or other times (CORK 1362A or CORK 1362B in 2011) because it costs them less energy to make AAs indicating when there is more reducing power available (Figure 3). Temporal changes in hydrogen concentration are explained by a fracture-controlled flow pattern (Sherwood Lollar et al.,

2006) while spatial variation is likely due to heterogeneity of basaltic basement (Lin et al., 2014). The AA synthesis reactions from HCO_3^- , NH_4^+ , H_2 , and HS^- (Table 1) in the Juan de Fuca basement fluids are endergonic (positive ΔG_r at the prevailing conditions, vertical dashed lines in Figure 3); therefore, the abiotic production of AAs is not favorable. In contrast, the synthesis of 8 out of the 14 protein-forming AAs is thermodynamically favorable (exergonic) under hot (100°C) and reducing deep submarine hydrothermal fluid conditions (Amend and Shock, 1998), suggesting that abiotic production may be a source of AAs in these hydrothermal systems (Shock, 1990; Amend and Shock, 1998; Shock and Canovas, 2010). The results presented in Fig. 3 are not surprising because the measured hydrogen concentrations in ridge-flank basement fluids (0.05-1.1 μM , Lin et al., 2012, Lin et al., 2014) are two orders of magnitude lower than that ($\sim 340 \mu\text{M}$) used in the thermodynamic calculations by Amend and Shock (1998). As shown in Figure 3, higher hydrogen concentrations yield lower ΔG_r values, indicating more favorable energetics for AAs synthesis. Results from thermodynamic modeling also show that there is a large difference in the Gibbs energies required for the synthesis of different AAs, mostly due to the difference in the molecular structures of individual AAs. Although it may seem surprising that less energy is required to produce larger aromatic AAs such as phenylalanine and tryptophan than to produce smaller AAs such as glycine and serine per electron transferred, relatively more oxidized aromatic organic molecules require less energy to make from inorganic precursors than more reduced organic compounds when the carbon source is HCO_3^- (LaRowe and Van Cappellen, 2011).

4.2.3 Downward transport from sediment porewater

Enhanced biological productivity in deep sediments at site 1301A has been observed and was suggested to be supported by the supply of sulfate from the basement (Engelen et al., 2008). Enhanced biological productivity accompanied by decomposition of sedimentary particulate organic matter leads to DFAA concentrations in deep sedimentary porewater two-to-three orders of magnitude higher and DHAA concentrations 9-15 times higher than those in the ridge-flank basement fluids, respectively (Table A6). Such a concentration gradient provides a force for AA transportation from overlying sediment into the basement. Diffusional inputs of inorganic substrates—including ammonium and alkalinity—from overlying deep sediment to the 3.5 million year old basement on the eastern flank of Juan de Fuca ridge have long been suggested (Elderfield et al., 1999; Wheat et al., 2000; Lin et al., 2012; Wheat et al., 2013). A recent study indicated that deep sedimentary DOC depth-profiles on the eastern flank of Juan de Fuca ridge also exhibit a diffusional trend with minimum concentrations near the sediment-basement interface (Lin et al., in press). The fact that the lowest total dissolved amino acid concentration found in the porewater extracted from the deepest core sampled (222 m below seafloor and 9 m above basement-sediment interface) may suggest that there is a diffusional loss for dissolved amino acids into the basement (Table A6).

4.2.4 Heterotrophic consumption—inferred from amino-acid-based degradation indices

The microbial preference for DFAAs over DCAAs (Keil and Kirchman, 1991a; Middelboe et al., 1995) provides an explanation for the low DFAA/DCAA ratios found in basement fluids. In addition to this bias, heterotrophic microorganisms have been shown

to preferentially remove and transform some AAs over others (Dauwe et al., 1999; Tanaka et al., 2011). As a result, three independent AA-based indices were developed to quantify the extent to which DOM has been modified during heterotrophic activity: carbon-normalized amino yields; Degradation Index (DI); and non-protein-forming AA (γ -aminobutyric acid) percentages.

Carbon-normalized AA yield, which is defined as the percentage of organic carbon comprised by DHAA, has been suggested as a sensitive indicator for the early stage of diagenesis (Cowie and Hedges, 1994; Davis et al., 2009; Kaiser and Benner, 2009). This index is based on observations that AAs are preferentially utilized during microbial decomposition of marine organic matter (Lee et al., 2000; Amon et al., 2001; Lee et al., 2004). The carbon-normalized AA yields for the ridge-flank basement fluids (1.3-3.3%) are similar to or slightly higher than those for seawater DOC (0.25-1.8 %, Druffel et al., 1992; Benner, 2002; Kaiser and Benner, 2009) but at the low end of those for previously reported non-ridge-flank hydrothermal vent fluids (2-25%, Table 5). High carbon-normalized AA yields are often associated with high fractions of labile organic matter, such as fresh plankton biomass (35%, Kaiser and Benner, 2009) and fresh plankton DOM (21%, Davis et al, 2009). Thus, the high AA yields in the non-ridge-flank hydrothermal vent fluids suggest that the DOM in these systems is freshly produced with minimum alteration. In contrast, the very low AA yield in deep seawater is attributed to extensive heterotrophic processing of DOM (Kaiser and Benner, 2009). The slightly higher AA yields in basement fluids relative to those in deep seawater, in fact, also result from normalizing similar AA concentrations to much more depleted total DOC concentrations

(12 μM , [Lin et al. 2012](#)) than bottom seawater (36 μM), indicating that DOC is preferentially removed over AAs in the basement.

Unlike carbon-normalized AA yields, which entirely rely on DHAA concentrations, DI values (Section 3.2) are based on the DHAA composition of a sample compared to reference materials (here, marine-algae-derived DOM and seawater DOM). DI has been suggested to be a sensitive indicator for intermediate stages of diagenesis ([Davis et al., 2009](#)). The calculated DI values ([Figure 4](#)) follow the general observation that high and usually positive values of DI are associated with source organic matter (fresh marine-algae DOM), while extensively degraded (deep seawater) DOM is characterized by low and sometimes negative DI values ([Amon et al., 2001](#); [Yamashita and Tanoue, 2003](#); [Kaiser and Benner, 2009](#)). The calculated DI values of DOM in the basement fluids (- 1.6 to 1.6, [Table 4](#) and [Figure 4](#)) are all lower than the DI value of fresh algal-derived DOM (2.6), while the DI values for CORK 1362A-2011 samples (- 1.2 ± 0.04) and CORK 1301A-2011 samples (- 1.6 ± 0.02) are even lower than that of deep seawater (- 0.8). The low DI values suggest that basement fluids contain degraded AAs.

Non-protein-forming AAs, γ -aminobutyric acid and β -alanine, are produced from incomplete degradation (i.e., partial decarboxylation) of glutamic acid and aspartic acid, respectively ([Schroeder, 1975](#); [Whelan, 1977](#); [Lee and Cronin, 1982](#)). In particular, γ -aminobutyric acid has been suggested to be a sensitive indicator for advanced DOM diagenesis ([Cowie and Hedges, 1994](#); [Dauwe et al., 1999](#); [Tanaka et al., 2011](#)). Both γ -aminobutyric acid (0.6-18 nM) and β -alanine (0.6-1.1 nM) are enriched in CORK

1301A, 1362A, and 1362B samples collected in 2011 (Table 4), while γ -aminobutyric acid and β -alanine are not detected (<0.1 nM) in CORK 1301A-2009 and 2010 samples. The systematic trends observed in plots of the mole percentage of glutamic acid versus γ -aminobutyric acid (Figure 5a) support the notion that the degradation of glutamic acid is accompanied by the accumulation of γ -aminobutyric acid, which is also observed in other studies (Cowie and Hedges, 1994; Dauwe et al., 1999; Kaiser and Benner, 2009; Tanaka et al., 2011).

Despite the large uncertainties, organic carbon-normalized AA yields, DI values, and the γ -aminobutyric acid percentage all suggest that degradation states of DOM in basement fluid samples changed with location and time (Figure 5). The DOM in CORK 1362A-2011 and CORK 1301A-2011 samples were extensively degraded while the DOM in CORK 1362B-2011 samples were at the early to intermediate stage of degradation. CORK 1301A-2009 and CORK 1301A-2010 samples may contain less degraded DOM than seawater. The degraded DOM in the basement fluid samples suggests that heterotrophic activity occurs throughout the fluid flow paths.

4.2.5 Surface sorption

AAs in basaltic aquifer are susceptible to surface sorption because fluids flowing through basement interact with all of the minerals typically found in basalt. The degree to which there are electrostatic interactions between mineral surfaces and amino acids depends upon the types of minerals present and the charge of the AAs. Although the backbones of all AAs are zwitterionic in aqueous solutions, the functional groups (side-chains) can be positively or negatively charged depending on the pH of the solution and

the nature of the functional group ([Table 1](#)). As a result of the heterogeneity of AA functional groups, some amino acids preferentially adsorb or desorb to mineral surfaces ([Henrichs and Sugai, 1993](#); [Ding and Henrichs, 2002](#); [Lambert, 2008](#)). Based on theoretical and empirical data, previous studies showed that clay minerals such as kaolinite and smectite sorb positively charged basic amino acids (e.g., arginine) and tend to repulse negatively charged acidic amino acids such as glutamic and aspartic acid at neutral pH ([Aufdenkampe et al., 2001](#); [Tappin et al., 2010](#)). That is, the relative abundance of aspartic and glutamic acids and depletion of arginine in ridge-flank basement fluids could be explained by electrostatic interactions with negatively surface-charged secondary clay minerals ([Marescotti et al., 2000](#)) in the 3.5 million year old basaltic rocks. In addition, some amino acids that are relatively hydrophobic, such as phenylalanine, isoleucine and leucine, tend to sorb to particles ([Aufdenkampe et al., 2001](#)), corresponding to lower abundances in basement fluids.

The effect of sorption can be visualized on a plot of scores of principal component analysis (SPSS, unrotated factor solution) of AA compositions for basement fluids, deep sediment porewater, sediment ([Andersson et al., 2000](#)), and North Pacific deep seawater ([Figure 6](#)). The scores of principal component analysis distinctly separate aqueous samples from sediment samples, indicating that DHAA compositions in aqueous samples are very different from those in particulate sediment samples. Sorption can be used to explain the enrichment of glutamic acid and aspartic acid in aqueous samples and the enrichment of hydrophobic AAs in particulate sediment samples. In addition, because peptides containing non-protein forming AAs have low surface affinity and are thus preferentially retained in solution ([Aufdenkampe et al., 2001](#)), desorption from surfaces

also enhances the enrichment of γ -aminobutyric acid and β -alanine in the fluid phase. Because sorption enhances the retention of non-protein AA in solution, non-protein forming AAs can no longer be interpreted solely as a diagenetic parameter when making a comparison between dissolved and particulate phases. Nevertheless, the production of non-protein-amino acids is from partial heterotrophic degradation of protein-forming AAs in particulate or dissolved AA (Schroeder, 1975; Whelan, 1977; Lee and Cronin, 1982); the enrichment of NPAA is still linked to microbial activity (Aufdenkampe et al., 2001). Interestingly, CORK 1362A-2011, CORK 1362B-2011, CORK 1301A-2011, 1301A-2010 and 1301A-2009 scores from low (negative) to high (positive) values in principle component two (y-axis in Figure 6), which mimics the change in their DI values (Figure 5) and suggests that principle component two reflects the degradation states of the samples. This notion is also supported by the observation that surface (young, less degraded) sediment samples have more positive numbers than deep (old, more degraded) samples (Figure 6). In sum, AA compositions in the basement fluid samples can be explained by alteration processes including surface sorption, desorption and heterotrophic alternation.

4.3 Spatial and temporal variation of amino acids in the basement

Spatial and temporal variations in amino acid concentrations and compositions are likely a result of fluctuations in flow patterns and interactions between water, rock, and microorganism. The change (43-89 nM) is very small if we compare the values with non-ridge-flank hydrothermal fluid AA concentrations (e.g. 0.736-2.3 μ M, Lang et al., 2013). A similar range of DHAA concentrations was observed at the North Pacific time-series-

station ALOHA (22°45'N, 158°W), from 109 nM in 1991 (McCarthy et al., 1996) to 60 nM in 2001 (Kaiser and Benner, 2009). The drop in DHAA concentration could be larger because McCarthy et al. (1996) only analyzed the high-molecular-weight fraction (> 1000-Dalton) of the DOM while Kaiser and Benner (2009) analyzed total DOM. Although the samples discussed in this study were collected using different methods, from 60L-Tedlar bag sampler in 2009 and 2010 to 15-L foil-lined, gamma irradiated bag sampler in 2011, the sampler blanks do not show AA contamination that could account for the change in AA concentrations. Disturbance from drilling and installment of CORKs 1362A and 1362B during IODP Expedition 327 in October 2010 may be another cause for the change in AA concentrations at site 1301A; however, we would expect pumping of drilling fluids—mainly surface seawater—would introduce fresher DOC instead of more refractory DOC observed in our 2011 samples. In addition, the changing degradation status of DOM in basement fluids collected from site 1301A from 2009 to 2011 also imply that (heterotrophic) microbial activity is temporally dynamic, which is not inconsistent with a temporal variation in microbial community found at the same study site (Jungbluth et al., 2013).

Spatial variation in AA concentrations and compositions in fluids from sites 1301A, 1362A and 1362B is also consistent with spatial variations in H₂ and CH₄ concentrations (Lin et al., 2012; Lin et al., 2014). Interestingly, 1301A-2010 samples contained higher amounts of amino acids and hydrogen relative to the 1362A-2011 samples (Table 3), but the 1362A-2011 sample contained more degraded amino acids than 1301A-2010 sample. A possible explanation for this observation is that the basement environment might be changing from one that promotes autotrophy to one where heterotrophy is more favorable

from 1301A to 1362A. From an energetic perspective, the lower H₂ concentrations at 1362A decrease to such low levels that autotrophic reactions such as methanogenesis become endergonic (Lin et al., 2014).

5. Conclusions

There is likely a complex, dynamic cycling on AAs in basement fluids supported by the amounts of AAs present, DFAA/DHAA ratios, AA compositions, and AA-based degradation indices. The concentrations of DFAA and DCAA in basement fluids are at the low end of the range of values reported for non-ridge hydrothermal vent fluid samples, largely attributed to conditions (moderate temperature, neutral pH, low particulate organic carbon input) unfavorable to dissolved AA production through heat degradation and acid hydrolysis. DFAA and DCAA in basement fluids are likely a mixture of AAs from recharging seawater, in situ microbial production, and input from overlying sediment. The DHAA compositions are probably controlled by microbial consumption and sorption to mineral surfaces. In situ biological synthesis of amino acids is supported by the energy yield from chemolithotrophic reactions. In addition, three AA-based degradation indices—carbon-normalized AA yields, Degradation Index, and γ -aminobutyric acid percentage—indicate that active heterotrophic organic matter degradation is occurring in basement fluids. In terms of on-going research, the AA data provide a guide for examining environmental genomic data from the same basement fluid samples. For example, the presence of relatively abundant γ -aminobutyric acid (up to 18%) indicates amino acid cycling during basement fluid flow that could be corroborated by gene sequences responsible for this metabolic activity. Overall, the data presented in this study provide a glimpse into the biogeochemistry of organic carbon dynamics in the

541 deep subsurface and provide a foundation for exploring the microbiology of the oceanic
542 basement.

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Figure captions

Figure 1. (a) Bathymetry map showing Integrated Ocean Drilling Program (IODP) sites U1301, U1362, and U1363 on the eastern flank of the Juan de Fuca Ridge. The insert shows the location of the study sites, marked by an open square, relative to North America. (b) Schematic depiction of the relative distances and penetration depths of CORKs 1362A, 1362B, and 1301A.

Figure 2. Amino acid chromatograms of (a) AAH standards (Thermo Scientific Pierce) in deep seawater at concentration between 10 to 250 nM, color-coded (from low to high concentration): black, deep seawater from station ALOHA (22°45'N, 158°W); brown, 10 nM; dark blue, 25 nM; cyan, 50 nM; green, 75 nM; blue, 100 nM; and red, 250nM. (b) CORK 1362A hydrolyzed sample. Total of fifteen protein forming amino acids are shown in these chromatograms, including aspartic acid (ASP), glutamic acid (GLU), serine (SER), histidine (HIS), glycine (GLY), arginine (ARG), threonine (THR), alanine (ALA), tyrosine (TYR), methionine (MET), valine (VAL), phenylalanine (PHE), isoleucine (ILE), leucine (LEU), and lysine (LYS). The peak of a non-protein forming amino acid, γ -aminobutyric acid (GABA), is apparent in the CORK 1362A sample.

Figure 3. Energetics of the amino acid synthesis reactions listed in Table 1 at the in situ temperature (65°C) and pressure (270 bar). The activities of amino acids, H_2 , NH_4^+ , and bicarbonate and pH used in these calculations are taken from Table 3. Black dashed lines bracket the hydrogen activities from IODP holes 1362A, 1362B, and 1301A (Lin et al., 2014). Positive values of ΔG_r indicate that the reactions as written in Table 1, and under

the specified conditions, are not favored to progress to the right. The order of amino acids listed in the legend follows the order of high to low energy demands from top to bottom.

Figure 4. Degradation Index of dissolved organic matter. More negative values indicate greater degrees of degradation. Algal DOM values are from a 10-day degradation experiment using fresh marine-algal-derived DOM as a substrate (Amon et al., 2001). The amino acid compositions of the North Pacific surface seawater and 2000 m deep seawater are from Kaiseer and Benner (2009).

Figure 5. Scatter plots of (a) glutamic acids (GLU) versus γ -aminobutyric acid (GABA); (b) Degradation Index versus carbon-normalized amino acid yields (AA yield %OC); (c) Degradation Index versus γ -aminobutyric acid. Gray error bars are standard deviations (1 SD); no replicate samples were run for the CORK 1301A 2009 sample and the error bars for this sample are the average deviations of all other samples.

Figure 6. Principal component analysis for comparison of amino acid compositions in the Juan de Fuca eastern flank basement fluids, deep sediment porewater from IODP Site 1363D, sediment from ODP Site 1025 and 1028, which are 65 km and 55 km west-northwest of Site 1301, respectively (Andersson et al., 2000), and North Pacific time-series station ALOHA deep seawater (McCarthy et al., 1996; Kaiser and Benner, 2009). Gray arrows denote amino acid loadings.

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Table 1. Net reactions of amino acid synthesis. Log K is the logarithm of equilibrium constant (K) for the indicated reactions at 65°C, 270 bar and pH=7.4.

Properties	Amino acids	Net amino acid synthesis reactions										Log K	
Negatively charged (acidic) side-chain	Aspartate	4HCO ₃ ⁻	+	2H ⁺	+	NH ₄ ⁺	+	6H ₂	→	C ₄ H ₇ NO ₄ ⁻	+	9 H ₂ O	45.97
	Glutamate	5HCO ₃ ⁻	+	3H ⁺	+	NH ₄ ⁺	+	9H ₂	→	C ₅ H ₈ NO ₄ ⁻	+	11 H ₂ O	69.39
Nonpolar, aliphatic side-chain	Alanine	3HCO ₃ ⁻	+	2H ⁺	+	NH ₄ ⁺	+	6H ₂	→	C ₃ H ₇ NO ₂	+	7 H ₂ O	45.00
	Glycine	2HCO ₃ ⁻	+	1H ⁺	+	NH ₄ ⁺	+	3H ₂	→	C ₂ H ₅ NO ₂	+	4 H ₂ O	19.07
	Isoleucine	6HCO ₃ ⁻	+	5H ⁺	+	NH ₄ ⁺	+	15H ₂	→	C ₆ H ₁₃ NO ₂	+	16 H ₂ O	123.23
	Leucine	6HCO ₃ ⁻	+	5H ⁺	+	NH ₄ ⁺	+	15H ₂	→	C ₆ H ₁₃ NO ₂	+	16 H ₂ O	124.48
	Valine	5HCO ₃ ⁻	+	4H ⁺	+	NH ₄ ⁺	+	12H ₂	→	C ₅ H ₁₁ NO ₂	+	13 H ₂ O	97.92
Polar, positively charged side-chain	Arginine	6HCO ₃ ⁻	+	3H ⁺	+	4NH ₄ ⁺	+	11H ₂	→	C ₆ H ₁₅ N ₄ O ₂ ⁺	+	16 H ₂ O	58.60
	Histidine ^a	6HCO ₃ ⁻	+	3H ⁺	+	3NH ₄ ⁺	+	10H ₂	→	C ₆ H ₉ N ₃ O ₂	+	16 H ₂ O	63.83
Polar, uncharged side-chain	Serine	3HCO ₃ ⁻	+	2H ⁺	+	NH ₄ ⁺	+	5H ₂	→	C ₃ H ₇ NO ₃	+	6 H ₂ O	29.09
	Threonine	4HCO ₃ ⁻	+	3H ⁺	+	NH ₄ ⁺	+	8H ₂	→	C ₄ H ₉ NO ₃	+	9 H ₂ O	53.54
Sulfur containing	Methionine	5HCO ₃ ⁻	+	5H ⁺	+	NH ₄ ⁺	+	11H ₂ + HS ⁻	→	C ₅ H ₁₁ NO ₂ S	+	13 H ₂ O	92.00
Aromatic side-chain	Phenylalanine	9HCO ₃ ⁻	+	8H ⁺	+	NH ₄ ⁺	+	20H ₂	→	C ₉ H ₁₀ NO ₂	+	25 H ₂ O	174.00
	Tryptophan	11HCO ₃ ⁻	+	9H ⁺	+	2NH ₄ ⁺	+	23H ₂	→	C ₁₁ H ₁₂ N ₂ O ₂	+	31 H ₂ O	193.67
	Tyrosine	9HCO ₃ ⁻	+	8H ⁺	+	NH ₄ ⁺	+	19H ₂	→	C ₉ H ₁₁ NO ₃	+	24 H ₂ O	161.97

a. Although histidine is likely uncharged at pH 7.4, it has an aromatic imidazole side chain that dissociates near neutral pH and become positively charged.

Table 2. Compositions of basement fluids collected from CORK 1362A, 1362B and 1301A. Numbers in parenthesis are uncertainties in reproducibility. Dash (-) indicate no data.

Sample site		CORK 1362B ^a		CORK 1362A ^a		CORK 1301A ^b		Bottom seawater		Analytical uncertainty
		2011		2011		2008-2011		2008-2010		
Variables ^c	unit	Mg Corr.	(n=9)	Mg Corr.	(n=6)	Mg Corr.	(n=12)	-	(n=5)	
Temperature	°C	65 ^d	-	65 ^d	-	65	-	1.7	-	-
Pressure	bar	270 ^d	-	270 ^d	-	270	-	270	-	-
pH	-	7.3	-	7.5	-	7.4	(0.1)	7.7	(0.1)	(0.1)
Magnesium	mM	1.6	-	2.5	-	1.9	-	53.7	(0.3)	(0.5)
Calcium	mM	55.9	(0.3)	54.5	(0.1)	55.0	(1)	10.4	(0.03)	(0.2)
Sulfate	mM	18.8	(0.1)	18.8	(0.4)	18.0	(0.3)	28.4	(0.3)	(0.3)
Chloride	mM	552	(2)	548	(1)	552	(6)	541	(14)	(5.4)
Sodium	mM	462	(1)	461	(1)	468	(6)	467	(13)	(3)
Potassium	mM	6.4	(0.1)	6.5	(0.1)	6.2	(0.4)	10.2	(0.3)	(0.2)
Alkalinity	meq/L	0.47	(0.01)	0.58	(0.02)	0.45	(0.04)	2.48	(0.05)	(0.02)
Hydrogen	μM	0.08	(0.03)	0.05	(0.02)	1.1	(1.2)	0.0004 ^e	-	(0.0002)
Ammonium	μM	101	(0.4)	99	(1.0)	102	(3.0)	<0.05	-	(0.1~2)
Sulfide	μM	0.17 ^d	-	0.17 ^d	-	0.17	-	-	-	-

a. This study and Lin et al. (2014)

b. Data from Lin et al. (2012)

c. All values are Mg corrected. See Lin et al. (2012) for details.

d. Data not available, using values for CORK 1301A fluids.

e. Data from Kelley et al. (1998)

Table 3. Activities of chemical species used for thermodynamic calculations. The proton, bicarbonate and ammonium concentrations are the average of 1301A, 1362B and 1362A values. The amino acid concentrations are the value or the average value of detected dissolved free amino acids in the samples collected from Hole U 1301A, 1362B and 1362A in Table 3. The detection limits are used for the undetected free amino acids such as phenylalanine and leucine.

Species	Detection limit (μM)	Concentration (μM)	Activity coefficient	Activity	$\log(\text{Activity})$
H^+	N.A.	0.051	0.80	4.1E-08	-7.4
HCO_3^-	0.2	359	0.67	2.4E-04	-3.6
NH_4^+	0.05	101	0.59	5.9E-05	-4.2
HS^-	0.15	0.17	0.64	1.1E-07	-7.0
H_2 _1301A-2010 ^a	0.0002	1.1	1.13	1.2E-06	-5.9
H_2 _1362A-2011 ^b	0.0002	0.05	1.13	5.7E-08	-7.2
H_2 _1362B-2011 ^b	0.0002	0.08	1.13	9.0E-08	-7.0
	Detection limit (nM)	Concentration (nM)			
ASP	0.2	1.1	0.66	6.9E-10	-9.2
GLU	0.2	1.2	0.66	7.9E-10	-9.1
SER	0.2	2.7	1.0	2.7E-09	-8.6
HIS	0.8	1.5	1.0	1.5E-09	-8.8
GLY	0.8	2.3	1.0	2.3E-09	-8.6
THR	0.3	0.3	1.0	2.7E-10	-9.6
ARG	0.2	1.0	0.66	6.6E-10	-9.2
ALA	0.2	1.0	1.0	9.5E-10	-9.0
TYR	0.2	0.4	1.0	3.5E-10	-9.5
MET	0.2	1.1	1.0	1.1E-09	-9.0
VAL	0.2	0.2	1.0	1.5E-10	-9.8
PHE	0.2	0.2	1.0	2.1E-10	-9.7
ILE	0.1	0.1	1.0	1.4E-10	-9.9
LEU	0.2	0.2	1.0	1.9E-10	-9.7

a. Data from Lin et al. 2012

b. Data from Lin et al. 2014

Table 4. Concentrations of dissolved free amino acids (DFAA), total dissolved hydrolysable amino acids (DHAA), dissolved organic carbon (DOC) and carbon-normalized amino acid yields (AA yield %OC) and degradation index based on DHAA for subseafloor basement fluids. Numbers in parenthesis are standard deviations (1SD) of analysis of replicate samples (n=2-5). There is only one CORK 1301A-2009 sample for DHAA analysis and thus no SD reported.

(nM)	CORK 1301A						CORK 1362 B		CORK 1362A	
	2009		2010		2011		2011		2011	
	DFAA (n=5)	DHAA (n=1)	DFAA (n=5)	DHAA (n=2)	DFAA (n=4)	DHAA (n=3)	DFAA (n=4)	DHAA (n=3)	DFAA (n=2)	DHAA (n=2)
ASP	1.5 (0.2)	14	- -	15 (0.0)	0.6 (0.1)	10 (2)	- -	9 (3)	- -	2 (1)
GLU	- -	13	0.9 (0.5)	15 (2.2)	1.5 (1.4)	8 (3)	- -	12 (2)	- -	5 (1)
SER	6.0 (1.3)	16	1.7 (1.1)	13 (0.7)	0.5 (0.2)	- -	- -	13 (0.6)	- -	7 (1.1)
HIS	0.8 (1.1)	1	- -	1 (0.0)	2.1 (0.8)	4 (2)	- -	1 (1)	- -	3 (0)
GLY	2.4 (1.3)	19	0.5 (0.5)	17 (4.2)	1.9 (1.0)	4 (5)	0.96 (0.7)	14 (2)	- -	17 (5)
THR	- -	5	1.2 (0.8)	7 (0.6)	- -	- -	- -	1.4 (1.2)	- -	1.7 (0.6)
ARG	- -	3	1.3 (1.0)	3 (1.3)	0.7 (0.8)	- -	- -	2.5 (0.8)	0.3 (0.1)	0.2 (0.4)
ALA	2.4 (0.7)	5	0.9 (1.3)	2 (0.9)	0.2 (0.2)	2.1 (2)	0.31 (0.1)	4 (3)	0.5 (0.0)	3 (0.5)
TYR	0.2 (0.2)	2	- -	2 (0.9)	- -	0.5 (0.5)	- -	1.1 (0.4)	- -	0.4 (0.4)
MET	- -	-	1.9 (1.6)	- -	0.3 (0.1)	2.3 (1)	- -	0.3 (0)	- -	2 (0.2)
VAL	0.7 (0.9)	4	- -	4 (1.8)	- -	0.9 (1)	- -	1.7 (1)	- -	0.8 (1)
PHE	- -	2	- -	2 (0.9)	- -	- -	- -	0.2 (0.3)	- -	0.1 (0.1)
ILE	0.5 (0.3)	-	- -	- -	- -	- -	- -	1.1 (0.3)	- -	0.1 (0.1)
LEU	- -	8	- -	8 (6.7)	- -	3.5 (2)	- -	1.0 (1)	- -	- -
LYS	- -	-	- -	- -	- -	- -	- -	- -	- -	- -
GABA	- -	-	- -	- -	- -	6 (2)	- -	0.6 (0.6)	- -	18.0 (0.4)
BALA	- -	-	- -	- -	- -	1.1 (0)	- -	0.6 (0.6)	0.3 (0.0)	- -
Total AA (nM)	14 (6)	89	9 (7)	88 (20)	8 (5)	43 (22)	1 (1)	65 (17)	1 (0)	60 (12)
DOC (µM-C)		13		11 (3)		12 (1)		11 (1)		16 (3)
AA yield (%OC)		2.8		3.3 (0.2)		1.7 (0.8)		2.2 (0.7)		1.3 (0.3)
Degradation Index		0.7		1.6 (0.2)		-1.2 (0.0)		-0.1 (0.6)		-1.6 (0.0)
DFAA/DHAA		0.16		0.10 (0.08)		0.18 (0.14)		0.02 (0.01)		0.02 (0.005)

Table 5. Dissolved free amino acids (DFAA), dissolved hydrolysable amino acids (DHAA) concentrations, carbon-normalized amino acid yields (AA yield % organic carbon), dissolved organic carbon (DOC), temperature and pH for hydrothermal fluids and hydrothermal-influenced sediment porewater in various hydrothermal systems.

Sample type and location	Temperature (°C)	pH	DFAA (μM)	DHAA (μM)	DOC (μM)	DHAA yield (%OC)	Reference
<i>Oceanic basaltic basement fluids</i>							
Juan de Fuca eastern flank (3.5 Ma basement)	65	~7.4	0.001-0.014	0.043-0.089	12-16	1.3-3	This study
<i>Deep submarine hydrothermal vents</i>							
Mid-Atlantic Ridge 30°N (Lost City)	~90 ^a	9-11 ^a	N.A.	0.736-2.3	~100	2.5-15	Lang et al. (2013)
Mid-Atlantic Ridge 15°N (Logatchev) and 4-9°S	~350-400	~3.5 ^b	0.038-0.377 (?)	N.A.	N.A.	N.A.	Klevenz et al. (2010)
Suiyo Seamount, Izu-Bonin Arc, NW Pacific (water depth ~1400m)	300	N.A.	N.A.	0.067-1.082 ^c	N.A.	N.A.	Horiuchi et al. (2004)
Guaymas Basin (water depth ~2000m)	152-319	N.A.	<0.001	N.A.	N.A.	N.A.	Haberstroh and Karl (1989)
<i>Shallow submarine hydrothermal vents (~1 m deep)</i>							
Baia di Levante, Vulcano, Italy	88	5.6	1.33-1.92	0.88-1.86	56-72	10-15	Svensson et al. (2004)
<i>Geothermal wells</i>							
Vulcano, Italy	42-81	2-6.3	0.33-78	0.39-35	34-1780	2-25	Svensson et al. (2004)
<i>Subterranean hydrothermal vent</i>							
Toyoha mine, Hokkaido, Japan	48-71	5.8-6.6	N.A.	2.6-6.1	N.A.	N.A.	Takano et al. (2003)
<i>Hydrothermal-influenced marine sediment porewater</i>							
Juan de Fuca eastern flank (sample depth: 207-222m)	N.A.	7.1-7.5	0.27-1.46	0.8-3.1	103-229	2.7-6.9	This study
Guaymas Basin (sample depth: 0-12 cm)	5-100	N.A.	5-446	N.A.	N.A.	N.A.	Haberstroh and Karl (1989)
Shallow submarine near Japan (sample depth: 0-30 cm)	80-104	7.2-7.5	22-196	98-338	2742-4942	15-23	Hoaki et al. (1995)
<i>Deep seawater (>1000m)</i>							
North Pacific Ocean (22°45'N, 158°W)-year 2001	N.A.	N.A.	N.A.	0.05-0.06	36-38	0.4-0.5	Kaiser and Benner (2009)
North Pacific Ocean (22°45'N, 158°W)-year 1991 ^d	N.A.	N.A.	N.A.	0.109	41	0.51	McCarthy et al. (1996)
North Pacific Ocean (31°N, 159°W)	N.A.	N.A.	0.2-1.1	0.09-0.159	34-36	0.9-1.6 ^e	Druffel et al. (1992)
North Pacific Ocean (2°N, 101°W; 0°S, 85°W)	N.A.	N.A.	~0	0.08-0.18	N.A.	N.A.	Lee and Bada (1975)

N.A.: Not available.

a: Temperature and pH derived from Lang et al. (2010).

b: pH derived from Schmidt et al. (2007).

c: End-member fluid compositions calculated based on data from Horiuchi et al. (2004)

d. McCarthy et al. reported only the ultrafiltered fraction (>1000 Dalton) of the dissolved organic carbon.

e. AA yield calculated based on an average of 3.8 carbon per amino acid.

Figure 1.

(Color version is for on-line publication. Black and white in print)

Figure 2.

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Figure 3.

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Figure 4.

Figure 5.

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Figure 6.