THE STABILITY OF AMINO ACIDS AT SUBMARINE HYDROTHERMAL VENT TEMPERATURES

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Abstract. It has been postulated that amino acid stability at hydrothermal vent temperatures is controlled by a metastable thermodynamic equilibrium rather than by kinetics. Experiments reported here demonstrate that the amino acids are irreversibly destroyed by heating at 240 °C and that quasi-equilibrium calculations give misleading descriptions of the experimental observations. Equilibrium thermodynamic calculations are not applicable to organic compounds under high-temperature submarine vent conditions.

Introduction

The first systematic investigations of the decomposition of amino acids as a function of temperature were carried out nearly four decades ago (Abelson, 1954, 1956; Conway and Libby, 1958; Vallentyne, 1964). A major decomposition reaction of many amino acids was found to be a decarboxylation yielding amines:

$$RR'(NH_2)COOH \rightarrow RR'NH_2 + CO_2$$

R and *R'* are the substituents on the α-carbon. One of the more thoroughly studied decarboxylation reactions is that of alanine, which produces ethylamine (for summary, see Bada, 1991). Based on measurements between 90 and 250 °C, the decarboxylation half-life of alanine is estimated to be only a few minutes at the 350 °C temperatures measured in the hydrothermal discharges of submarine vents.

For some amino acids, other reactions besides decarboxylation also take place. For example, aspartic acid undergoes a reversible deamination reaction producing fumaric acid and ammonia (Bada and Miller, 1970). The decomposition of serine is complex and involves three reactions (Friedmann *et al.*, 1970; Bada *et al.*, 1978): decarboxylation to ethanol amine; dehydration, first to pyruvic acid and ammonia, followed by transamination or reduction to alanine; and reversible dealdolization to glycine and formaldehyde.

As part of an evaluation of whether submarine hydrothermal vents were important in the origin of life, we heated an aqueous mixture of aspartic acid, alanine, serine, and leucine at 250 °C and 250 atm (Miller and Bada, 1988). Substantial decomposition of aspartic acid and serine took place even before the samples reached the 250 °C reaction temperature. Glycine, which was not initially present in the reaction mixture, was produced as expected from the decomposition of serine, as clearly stated in our paper. Alanine was also produced from the serine. The

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results confirmed that amino acid decomposition at the temperatures characteristic of submarine hydrothermal vents is rapid. This high temperature instability of amino acids is consistent with the observations that there are no detectable amounts ($<10^{-9}$ M) of amino acids in the 319 °C Guaymas Basin hydrothermal vent waters (Haberstroh and Karl, 1989).

Using thermodynamic based calculations, Shock (1990a, b) has postulated that a quasi-equilibrium was reached during our 250 °C experiment and as a result, significant concentrations of amino acids could be present in hydrothermal vent waters even though they are unstable at high temperatures. According to these theoretical calculations, amino acid stability and concentrations in geochemical systems are determined not by kinetics but rather by metastable thermodynamic equilibria (MTE) governed by redox conditions (Shock 1990a, b). MTE calculations have been used to conclude that the organics on the early Earth could have been supplied by hydrothermal vents (Shock, 1992), and to bolster earlier claims that the origin of life took place in hydrothermal environments (Corliss *et al.*, 1981; Holm, 1992). However, even the H_2 concentration in hydrothermal vent waters is far less than that predicted by thermodynamic equilibrium calculations and is apparently not controlled by the quartz-fayalite-magnetite (QFM) redox buffer (Lilley *et al.*, 1982).

There is a considerable literature in which thermodynamic equilibrium based calculations have been used to estimate the concentrations of organic compounds produced from inorganic components under geochemical conditions (for example, see Suess, 1962; Dayhoff *et al.*, 1964; Eck *et al.*, 1966; Lippincott *et al.*, 1967; Thorstenson, 1970). Although these calculations may be mathematically correct, the predicted equilibrium concentrations are not observed since equilibrium among organic compounds is rarely attained (Miller and Bada, 1991). Catalysts that can achieve favorable equilibria at low temperatures are not known.

According to the MTE calculations of Shock (1990a, b) the relative concentrations of alanine, glycine and leucine at 250 °C in aqueous solutions should be determined by the following equilibrium and its K_{eq}:

$$\underset{\text{alanine}}{4C_3H_7NO_2} \rightleftarrows \underset{\text{glycine}}{3C_2H_5NO_2} + \underset{\text{leucine}}{C_6H_{16}NO_2} \qquad (1)$$

$$K_{eq} = (\text{gly})^3(\text{leu})(\text{ala})^{-4} = 1.3 \times 10^{-3}$$

The selection of alanine as the sole reactant, and glycine and leucine as the only products, is arbitrary considering the large number of possible isomeric molecules, many of which have similar free energies of formation (Shock and Helgeson, 1990). In the case of the six carbon α-amino acids, such as leucine, there are 7 other isomeric α-amino acids (isoleucine, alloisoleucine, norleucine, 3,3-dimethyl-α-aminobutyric acid, 2-methylnorvaline, 2,3-dimethyl-α-aminobutyric acid, 2-ethyl-α-aminobutyric acid). In addition, there are over 200 other isomers of leucine, not counting optical isomers.

We have examined here the above proposed MTE involving alanine, glycine and leucine under simulated hydrothermal conditions. We show that no equilibrium, quasi or otherwise, involving these three amino acids would be attained at hydrotheral vent temperatures. If equilbrium among leucine, glycine and alanine is to be claimed, then surely the other 6-carbon α-amino cids should be formed, as well as some of the other isomers. No isomers of leucine were detected.

Experimental

Phosphate buffered solutions (ionic strength = 0.5) of alanine and glycine + leucine (molar ratio = 2:1), and ethylamine were heated at 240 °C. The pH of the solutions at 25 ° was adjusted to 7.00; the pH at 240 ° was calculated to be 8.48 using the temperature dependence of pK_2 of phosphoric acid (Robinson and Stokes, 1959). Aliquots were placed in glass tubes, degassed, and then sealed under vacuum. The glass tubes were placed in a water filled (to equalize the pressure) Teflon-lined steel tubes which were sealed with a steel cap. The samples were heated in a gas chromatograph oven at 240 °C for various lengths of time. A duplicate set of samples to which the mineral mixture quartz-fayalite-magnetite (QFM) was added to buffer the redox conditions were heated simultaneously. After heating, the amino acid and amine composition of the mixtures were determined by HPLC with OPA derivation using the procedure described elsewhere (Zhao and Bada, 1989). Standards of the amino acids and the expected amine decomposition products were run along with the heated samples in order to verify peak identifications.

1. Results and Discussion

Figure 1 shows the results of the HPLC/OPA analyses of the heated solutions which initially contained only L-alanine (Figure 1, bottom), and a 2:1 molar ratio of glycine and D/L-leucine (Figure 1 top). The main decomposition products were confirmed to be amines derived from the starting amino acids. The relative decomposition rates of alanine, glycine and leucine were found to be 1.0:0.86:2.1, which is consistent with prior measurements (Vallentyne, 1964). It should be noted that the racemization rate of alanine is more rapid than its rate of decomposition. Glycine and leucine, as well as other possible 6 carbon amino acids, were not detected in the heated alanine solutions. Likewise, alanine was not detectable in the heated solutions of glycine and leucine.

It was subsequently claimed by Shock (1990b) that the experiments need to be buffered at the oxygen fugacity of the vents, but the equilibrium of Equation (1) does not involve an oxidation or reduction. The oxygen fugacity might affect a kinetic process, but not this thermodynamic equilibrium. In any case, there is no reasonable mechanism for oxygen fugacity to affect a decarboxylation reaction.

The decomposition rates of alanine were found to be the same in the experiments with and without the QFM redox buffer (Figure 2). Ethylamine was found

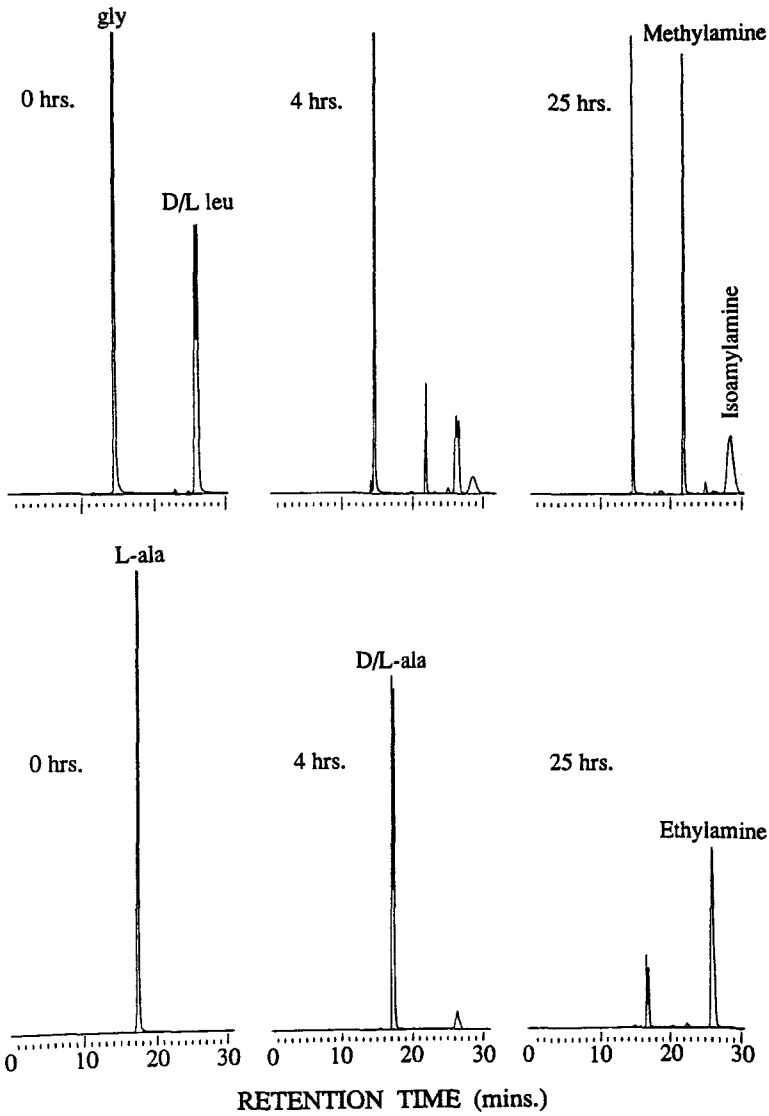

Fig. 1. HPLC chromatograms of L-alanine (bottom) and glycine and *D/L*-leucine (top) heated at 240 °C. Also shown is the unheated solution.

to decompose much more slowly than its parent alanine (Figure 2) which explains why it was still present in the heated solutions after nearly all of the alanine had decomposed. The decomposition products of ethylamine were not investigated but may be acetaldehyde, H_2 and NH_3. The results in Figure 2 show that the decomposition of both alanine and ethylamine follow irreversible first-order kinetics.

Using the K_{eq} value given above for Equation (1), we have calculated in Table I the amino acid concentrations predicted by MTE in the solutions heated for 25 h

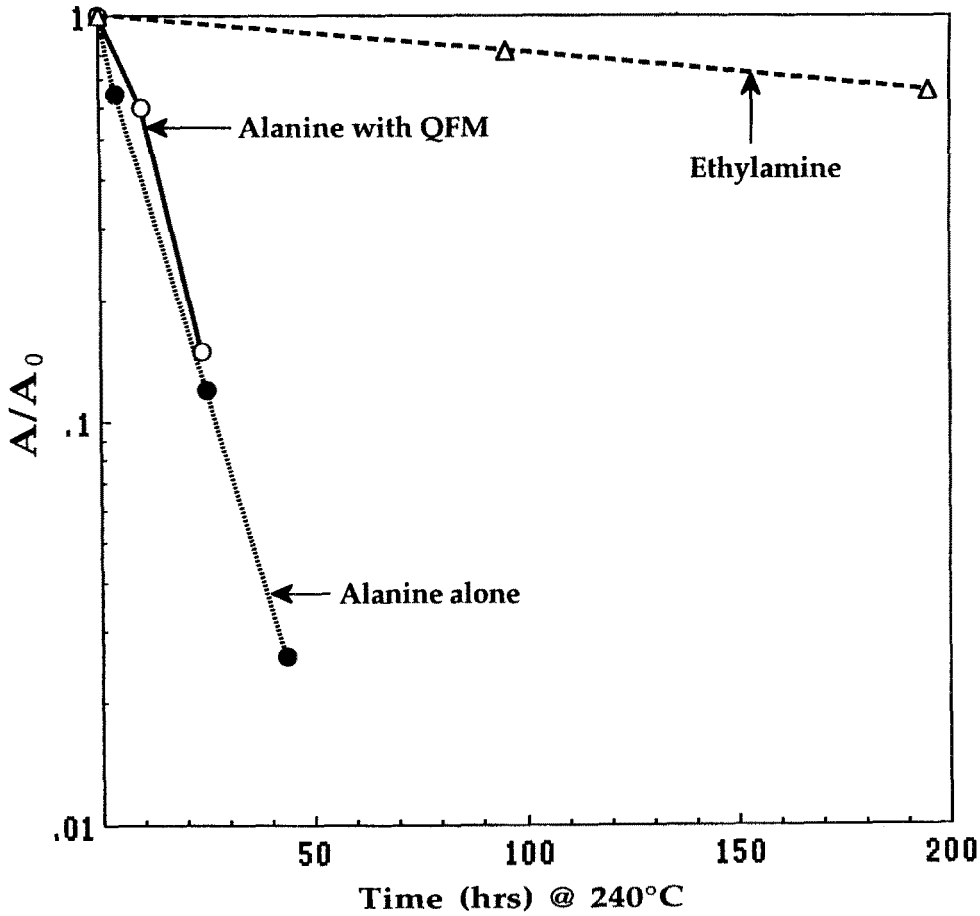

Fig. 2. Time course of decomposition at 240 °C of alanine, with and without QFM redox buffer, as well as ethylamine.

at 240 °C and compared these with the measured amino acid concentrations. In the heated solution of alanine, the glycine and leucine concentrations were less than 10^{-2}% of the MTE predicted concentrations. For the solution initially containing glycine and leucine, the alanine concentration was determined to be less than 10^{-4}% of that predicted on the basis of MTE calculations. These experimental results demonstrate that the stabilities and relative concentrations of alanine, glycine and leucine are not controlled by MTE under simulated hydrothermal vent conditions.

Of equal importance is the fact that no isoleucine, norleucine, nor any other isomer of leucine was produced in these experiments, although many of these would have been easily detected with our analytical system. If MTE were attained with alanine, glycine and *leucine*, then it would also be attained with alanine, glycine and *isoleucine* since the same number of carbon-carbon bonds are made and broken. The same argument applies to many of the other >200 isomers of

TABLE I

Amino acids concentrations (M) in solutions heated at 240 °C. The calculated concentrations are based on the K_{eq} value given for Equation (1), and the assumption that glycine and leucine are in equilibrium with the final measured concentration of alanine in the first case, and that alanine is in equilibrium with the final measured concentrations of glycine and leucine in the second case.

	ala_{obs}	ala_{calc}	gly_{obs}	gly_{calc}	leu_{obs}	leu_{calc}
t=0 h	0.2					
t=25	0.024		$<10^{-7}$	6×10^{-3}	$<10^{-7}$	2×10^{-3}
t=0			0.10		0.05	
t=25	$<10^{-7}$	0.04	0.016		6×10^{-4}	

leucine. In other words, if MTE is assumed for one isomer of an organic compound, then equilibrium must be assumed for all its isomers unless there is experimental evidence or a valid theoretical reason that equilibrium is not attained.

The rate of decomposition of ethylamine was found to be about 40 times slower than that of alanine at 240 °C. Ethylamine has been tentatively detected in 319 °C hydrothermal vent waters in the Guaymas Basin (Haberstroch and Karl, 1989). Because amine decomposition rates are much slower in comparison to the parent amino acids, it is possible that amines could survive passage of seawater through submarine hydrothermal vents. By accurately measuring the rate and temperature dependence of amine decomposition and determining the amine concentrations in vent waters, it should be possible to estimate the time and temperature at which dissolved organics such as amino acids and their decomposition products were heated during the circulation of seawater through hydrothermal vent systems.

Conclusions

Our results indicate that MTE regulated by redox conditions are not important in determining the stability and concentrations of amino acids at the >350 °C temperatures characteristic of hydrothermal vents at oceanic ridge crests. This is true whether the oxygen fugacity is unregulated or controlled by the QFM buffer. Amino acids are irreversibly destroyed during ocean circulation through hydrothermal environments although their amine decomposition products may remain if the contact time at high temperatures is not too long. The circulation of the oceans through hydrothermal systems on the Earth is an important sink at the present time for amino acids, not a source. This would have also been the case on the early Earth (Stribling and Miller, 1987).

The experiments reported here demonstrate again the problems with thermodynamic equilibrium calculations involving organic compounds. Organic reactions that involve breaking and reforming carbon-carbon bonds rarely come to equilibrium below temperatures of about 700 °C. Some reactions, such as dehydrogenations, ester formation from the acid and alcohol, and dehydration of alcohols achieve a quasi-equilibrium at lower temperatures, but no carbon-carbon bonds are broken in these reactions.

The inability of organic compounds to come to thermodynamic equilibrium is a necessity for the existence of life, since all organic compounds are unstable with respect to decomposition to graphite, CO_2, N_2, H_2 etc. If equilibrium were achieved then organic polymers which make up living organisms could not exist.

Although the assumption of thermodynamic equilibrium generally leads to a good description of inorganic systems, such calculations usually lead to misleading conclusions for organic mixtures. Thermodynamics predicts which reactions are possible and which are impossible. It does not say which reactions actually take place. This is especially pertinent to organic reactions where thermodynamically permissable reactions rarely occur.

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