CHAPTER 8

AN EXPERIMENTAL APPROACH TO CHEMICAL EVOLUTION IN SUBMARINE HYDROTHERMAL SYSTEMS

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1. Introduction

The discovery of submarine hydrothermal vents in the late 1970's (Corliss *et al.*, 1979) stimulated geophysical, geochemical, microbiological, ecological and ore deposit research on submarine hydrothermal systems. Since these systems represent reducing, energy-rich, and metal ion-rich conditions in the present terrestrial environment, they are considered to be ideal sites for present-day abiogenic synthesis of organic compounds and have been suggested as a possible environment for chemical evolution toward the origin of life (Corliss *et al.*, 1981; Baross and Hoffman,1985). However, it has also been suggested before the discovery of submarine hydrothermal vents that an important relationship may exist between the evolution of the Earth's crust such as a hydrogeothermal zone associated with axes of plate spreading and the process of chemical evolution (Ingmanson and Dowler, 1977; Degens, 1979).

From the point of view of chemical evolution, reducing environments are attractive because amino acids can be synthesized abiotically from reduced gas mixtures with spark discharges (Miller, 1953), heat (Harada and Fox, 1964), ultraviolet light (Sagan and Khare, 1971), and shock waves (Bar-Nun *et al.*, 1970). These experiments demonstrate that if the primitive earth atmosphere was reduced, consisting of a mixture of methane, ammonia and water, amino acids and other organic compounds could have been easily obtained with the available energy sources on the early Earth.

Recent studies on the formation of planets have suggested, however, that high velocity impacts of small bodies into a growing planet can result in impact-degassing of volatiles and formation of an impact-induced, high temperature atmosphere (Abe, 1986; Holloway, 1988; Kasting, 1990; Matsui and Abe, 1986a,b). According to this hypothesis, such an atmosphere contains carbon monoxide or carbon dioxide as a major carbon source. In addition, results of photochemical studies with an early Earth atmosphere show that the presence of hydroxyl radicals from the photodissociation of H_2O, together with the greater flux of UV radiation from the young sun, would have limited the half

Origins of Life and Evolution of the Biosphere **22**: 147–159, 1992.

lives of reduced gases such as methane and ammonia to about 50 years and about 1 week, respectively (Canuto *et al.*, 1982, 1983; Kasting *et al.*, 1983; Levine, 1982, 1985; Zahnle and Walker, 1982). Therefore, the primitive Earth atmosphere may have been only "mildly reduced". Some researchers view this as a problem for conventional chemical evolution theories because only traces of amino acids are obtained in simulated primitive atmosphere experiments when carbon monoxide or carbon dioxide are used as the carbon source (Schlesinger and Miller, 1983).

In a mildly reduced atmosphere, organic molecules required for the origin of life could be formed in local "reducing" environments like submarine hydrothermal systems, high latitude areas irradiated with cosmic rays and solar flare particles, and impact sites of comets and meteorites. Recently, Kobayashi *et al.* (1989, 1990) found that proton irradiation, simulating the action of cosmic rays and solar flare particles, formed imidazole and proteinaceous and nonproteinaceous amino acids such as glycine, alanine, aspartic acid, and β-alanine, from a mixture of carbon monoxide, carbon dioxide, nitrogen, and water. The production of organic compounds in the cooling gas mixtures formed during impacts has also been considered (Chyba *et al.*, 1990). The oxidation states prevailing during major impacts will be determined by the composition of the target, which in the case of the Earth would likely lead to oxidized conditions (Fegley *et al.*, 1986). Submarine hydrothermal systems may provide the type of reduced environment needed for the abiotic synthesis of various bioorganic compounds. In the present-day oceans, hydrothermal vents are more reduced than their immediate vicinity, since hydrothermal fluids contain reduced gases such as methane, hydrogen, hydrogen sulfide, and ammonia (Lilley *et al.*, 1983).

Although methane in the Earth's crust is formed biologically and abiologically from organic matter, it can also form at magmatic and metamorphic conditions from carbon dioxide and hydrogen (Wakita and Sano, 1983; Holloway, 1984; Schoell, 1988; Wakita *et al.*, 1990). There is also ample heat in submarine hydrothermal systems for driving abiotic synthesis reactions. MacDonald *et al.* (1980) calculated that the heat flow from a single chimney was equivalent to the total heat loss for a 3- to 6-km segment of spreading center out to 10 km on either side. Black smokers on the East Pacific Rise at 21°N operate at temperatures up to and above 350°C with high flow rates. Therefore, if heat can overcome kinetic barriers to the formation of metastable states, abiotic synthesis under hydrothermal conditions is a possibility (Shock, 1990b).

Submarine hydrothermal fluids contain high concentrations of metal ions such as iron, manganese, copper, and zinc (Edmond *et al.*, 1982; Von Damm *et al.*, 1983), which may be able to catalyze organic synthesis reactions, and which are essential to many present-day biochemical processes. The role of trace elements in chemical evolution has been emphasized by Egami (1974) who found that a close correlation exists between the concentrations of transition elements in contemporary sea water and their biological importance (Table 1). Egami (1974) suspected that transition elements relatively abundant

TABLE 1

Concentrations of transition elements in seawater and their biological importance

Concentration in seawater	Transition element	Biological importance
About 100-40 nM	Mo	Probably essential to all organisms including strict anaerobes
	Zn	Probably essential to all organisms including strict anaerobes
	V	Essential to a wide variety of organisms
	Fe	Essential to all organisms including strict anaerobes
About 40-10 nM	Ni	Probably essential to higher land animals and plants
	Ti	No evidence that it is a bioelement
	U	No evidence that it is a bioelement
	Cu	Essential to all organisms, probably with the exception of anaerobes
About 10-1 nM	Cr	Probably essential to higher land animals
	Mn	Essential to a wide variety of organisms including certain bacteria
	Cd	No evidence that it is a bioelement
	Co	Essential to a wide variety of organisms including certain bacteria
About 1-0.1 nM	W	W can replace Mo in several enzymes
	Ag	No evidence that it is a bioelement
	Zr	No evidence that it is a bioelement
	Hg	No evidence that it is a bioelement
	Nb	No evidence that it is a bioelement
	Ce	No evidence that it is a bioelement

in sea water such as molybdenum, iron, and zinc must have played important roles in the course of chemical evolution in the primeval sea, and proposed that the inorganic composition of the primeval sea was essentially similar to that of the present sea.

Based upon this idea, Egami, Yanagawa and coworkers have been trying an experimental approach to chemical evolution in the primeval sea. A "modified sea medium" was designed to accelerate possible chemical evolution reactions in the laboratory. It was enriched with 1,000-100,000 times higher concentrations of six transition elements: iron, molybdenum, zinc, copper, cobalt, and manganese. As mentioned above, it was found that amino acids and related compounds could be formed from formaldehyde and hydroxylamine (Hatanaka and Egami, 1977; Kamaluddin *et al.*, 1979), sugars and ammonia (Yanagawa *et al.*, 1980a, 1981), and carbon suboxide (C_3O_2, O=C=C=C=O) (Yanagawa and Egami, 1981) in the modified sea medium. Furthermore, it was found that marigranules, highly organized particles, were produced from a reaction of glycine and acidic, basic, and aromatic amino acids at 105°C in the modified sea medium (Yanagawa and

Egami, 1980; Yanagawa *et al.*, 1980b, 1988). We believed that metal ions in the modified sea medium played important roles in the formation of amino acids, peptides, and peptide-like polymers. When Egami and co-workers designed the modified sea medium enriched with high concentrations of six metal ions in 1973, they were unaware of a natural, near surface environment containing such high concentrations of metal ions. Submarine hydrothermal vents were discovered later and their metal ion concentrations are similar to those of the modified sea medium. Results of recent experiments using similar aqueous solutions are described in the next two sections.

2. Formation of Amino Acids under Simulated Submarine Hydrothermal System Conditions

A medium that approximated hydrothermal vent sea water, designated the modified hydrothermal vent medium (MHVM), contained 2mM $Fe(NH_4)_2(SO_4)_2$, 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. Its pH was adjusted to 3.6 with 1 N HCl. The metal ion concentrations and pH were set close to the reported value of hydrothermal vent sea water (Edmond *et al.*, 1982; Von Damm *et al.*, 1983). All water used was double distilled in glassware. All glassware was acid (7 M nitric acid) washed and rinsed repeatedly with double distilled water. All chemicals were analytical grade. The MHVM (30-50 ml) was put into a pyrex glass tube (22 mm x 190 mm), which was placed in a stainless steel autoclave (inner volume: 100ml). The autoclave was purged with nitrogen five times. A gas mixture of methane (40 kg/cm^2) and nitrogen (40 kg/cm^2) was then put into the autoclave at room temperature, which was heated at 325°C for 1.5-12 hours. After cooling to room temperature, the resulting aqueous solution was filtered through a membrane filter of 0.2 μm pore size, freeze-dried and hydrolyzed with 6 N hydrochloric acids for 24 hours. Amino acids were extracted from the acid-hydrolyzate with an AG-50WX8(Bio-Rad) resin column, and analyzed by ion-exchange chromatography (Irica Automated Amino Acid Analyzed Model A-5500), by gas chromatography (GC: Shimadzu Model GC-9A with a FID detector, column: Chirasil-Val capillary column) and by gas chromatography combined with mass spectrometry (GC/MS; Shimadzu Model GCMS-QP1000 column: Chirasil-Val capillary column).

Fig. 1 shows an ion exchange chromatrogram of (a) the amino acid fraction of the product compared to (b) a procedural blank. Amino acids such as glycine, alanine, aspartic acid, serine, and glutamic acid, together with non-proteinaceous amino acids (e.g., 2-aminobutyric acid and sarcosine) were detected in the chromoatogram. In other experiments, the yield of aspartic acid and glutamic acid increased when carbon dioxide was added to the starting reaction mixture. The major amino acid products: glycine, alanine, and sarcosine, were also identified by GC (Fig. 2) and/or GC/MS (Fig. 3). Evidence that these amino acids were synthesized abiotically from methane, includes: (1) the gas chromatographic analysis with an optically-active column indicated that the resulting

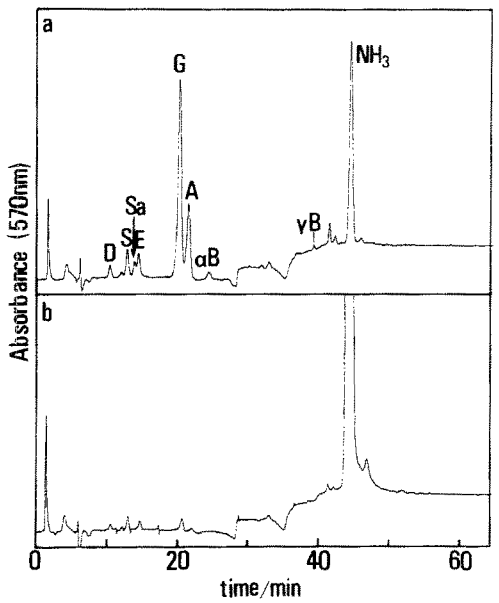

Fig. 1. Amino acid analysis of the products formed from methane and nitrogen in a modified hydrothermal vent medium by heating at 325°C and 200 kg/cm^2 for 1.5 hours (a) and a procedural blank (b) Abbreviations: D, aspartic acid; S, serine; Sa, sarcosine; E, glutamic acid; G, glycine; A alanine; αB, 2-aminobutyric acid; γB, 4-aminobutyric acid.

amino acids were racemic mixtures (Fig. 2); (2) non-proteinaceous amino acids such as sarcosine and 2-aminobutyric acid were detected (Fig. 1a); and (3) the procedural blank gave much lower amounts of amino acids (Fig. 1b). The yield of minor amino acids such as aspartic acid, serine, and glutamic acid were of the order of those in a procedural blank (Fig. 1a). Table 2 shows the yield of major amino acids synthesized in the present experiments. Larger amounts of glycine, alanine, and sarcosine were obtained when a longer heating period was applied. After the reaction, silica was found with Fe(III) and Mn(IV) in the precipitate. The silica was apparently derived from the pyrex glass wall. Effects of metal ions on the formation of amino acids was examined by removing metal ions from the MHVM, in which case the yield of amino acids was remarkably decreased. Some trace metals are now known to be essential to present-day life, and they may act as catalysts for prebiotic reactions in the course of chemical evolution (Kobayashi and Ponnamperuma, 1985).

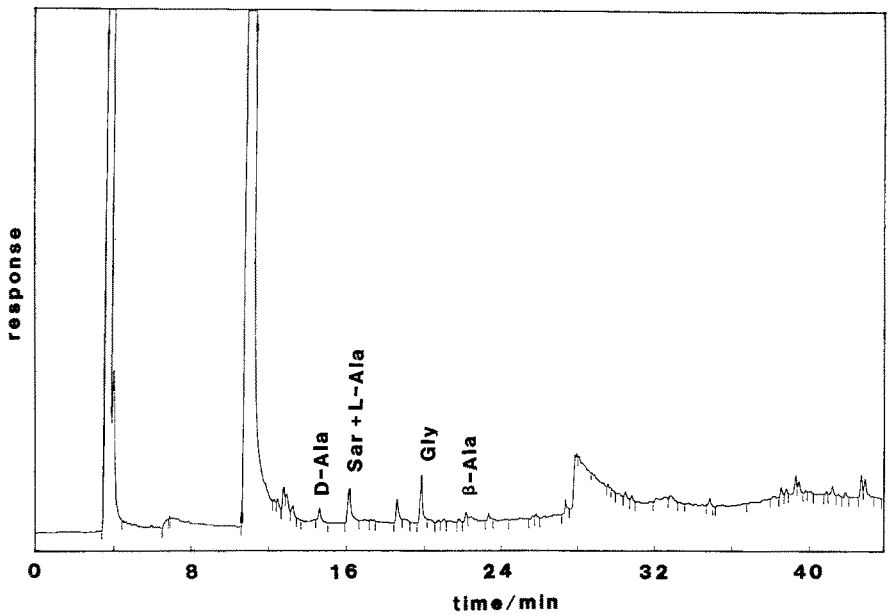

Fig. 2. Gas chromatogram of amino acids synthesized under hydrothermal vent condition. Reaction condition was the same as the run No. 3 in Table 2. Amino acids were derivatized with 2-propanol and N-trifluoroacetyl anhydride before injection.

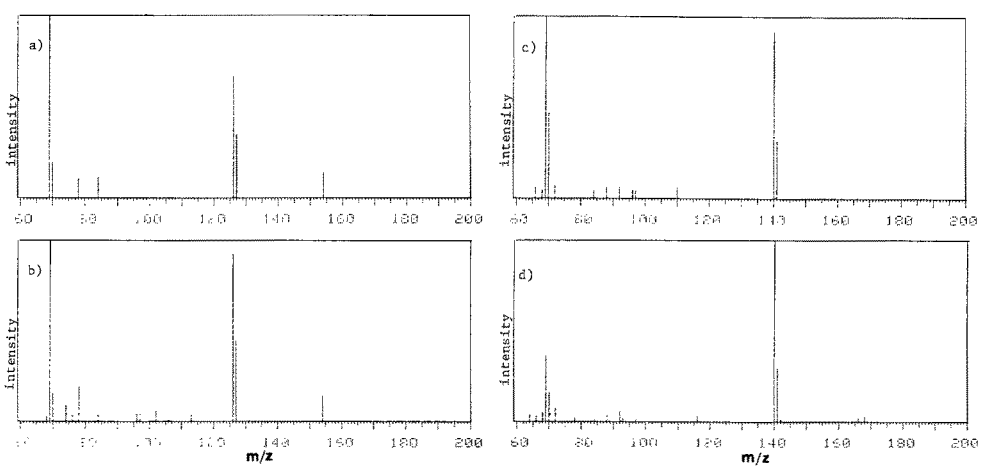

Fig. 3. Mass spectra of N-trifluoroacetyl amino acid 2-propylesters obtained with a gas chromatograph/mass spectrometer. (a) glycine standard; (b) glycine in the product; (c) D-alanine standard; (d) D-alanine in the product. Sample and gas chromatographic conditions were the same as those in Fig. 1.

TABLE 2

Formation of amino acids in superheated hydrothermal environments

			Reaction System			
Reaction condition	Procedural blank		1	2	3	4
Medium	MHVM	MHVM	H_2O	MHVM	MHVM	MHVM
Volume (ml)	40	40	50	50	50	30
CH_4 (kg/cm^2)	40	0	40	40	40	40
N_2 (kg/cm^2)	40	80	40	40	40	20
CO_2 (kg/cm^2)	0	0	0	0	0	12
Temperature (°C)	25	325	325	325	325	260
Pressure (kg/cm^2)	80	200	200	200	200	140
Time (h)	12	3	12	1.5	12	6
Major amino acids produced (pmol/ml)						
Glycine	54	47	57	1190	1990 (2.4×10^{-4})*	2400
Alanine	29	21	trace	600	750 (1.4×10^{-4})	630
Sarcosine	0	0	trace	640	1480 (2.7×10^{-4})	**

* The yield(%) on methane.

**Not separated from a large peak of glutamic acid.

The results summarized here support the possibility that amino acids such as glycine and alanine are produced abiotically in present-day submarine hydrothermal systems (Ingmanson and Dowler, 1980). The yield of amino acids was not high, because the experimental set up is a closed system, and amino acids synthesized in the thermal reaction would be thermally decomposed. If reactions were carried out in flow reactors like actual submarine hydrothermal systems (Corliss, 1986), the yield would be expected to increase since the products would be moved away from the threat of thermal decomposition.

3. Formation of Thermophilic Microspheres and Peptide-Like Polymers under Simulated Submarine Hydrothermal System Conditions

Experimental results summarized above indicate that abiotic synthesis of amino acids and other organic compounds from inorganic starting materials may be possible in submarine hydrothermal systems. Questions as yet unanswered concern the role of hydrothermal environments in the abiotic synthesis of polypeptides. Results summarized below show

that extremely thermophilic microspheres and peptide-like polymers can be formed in aqueous solution at 250-350°C.

The four amino acids glycine, alanine, valine, and aspartic acid have been used as starting materials for prebiotic synthesis of polypeptides (Yanagawa *et al.*, 1990; Sakurai and Yanagawa, 1984; Yanagawa, 1984). These amino acids were chosen because they are the more abundant of the amino acids formed in prebiotic synthesis experiments (Miller and Orgel, 1973), and are found in the Murchison meteorite (Kvenvolden *et al.*, 1970). Thus glycine, alanine, valine, and aspartic acid are likely to have been present in the early stages of chemical evolution (Eigen and Shuster, 1978; Shimizu, 1981; Egami, 1981).

When an aqueous solution (15 ml) containing 0.3 M glycine, 0.1 M L-alanine, 0.3 M L-valine, and 0.1 M L-aspartic acid was put in a glass tube and heated at 250°C and 134 atm for 6 hours, numerous microspheres were formed (Yanagawa and Kojima, 1985). These are organized particles of 1.5 to 2.5 μm in diameter. Fig. 4a shows the microspheres observed on scanning electron microscopy. The microspheres had a boundary layer structure whose thickness was about 30 nm (Fig. 4b). They were also obtained in reactions at 300°C, though their spherical structures were deformed (Fig. 4c and d).

Microspheres were not obtained in the reaction at 200°C. However, IR and NMR studies on the heated mixture revealed the presence of amide bonds and the near absence of imide bonds. After hydrolysis of products, glycine, alanine, valine, and aspartic acid were found in nearly equal amounts. When the reaction mixture was heated at 250°C and 134 atm for 6 hours in a stainless steel vessel, no microspheres were observed. Microspheres were not obtained from mixtures of glycine alone, of glycine-alanine or of glycine-alanine-valine heated at 250°C for 6 hours, suggesting that the presence of aspartic acid is crucial for microsphere production. Other polar amino acids such as glutamic acid, lysine, arginine, histidine, serine, threonine, and 4-hydroxyproline could be used instead of aspartic acids to form microspheres (data not shown). Especially, basic amino acids (lysine, arginine, and histidine) resulted in the formation of larger microspheres (4-8 μm in diameter). These results indicate that a polar amino acid, a glass tube, and a reaction temperature of 250°C or above are necessary for the formation of the microspheres.

Elemental analysis of the microspheres showed a percentage composition of C, 41.7; H, 4.3; N, 5.0; Si 12.0%; and others, e.g. oxygen and ash. The IR spectrum of the microspheres showed strong adsorption bands at 3400, 2970, 2940, 2870, 1670-1630, 1090, and 470 cm^{-1}. When the microspheres were treated with hydrofluoric acid, two clear bands appeared at 1670 and 1630 cm^{-1} that can be attributed to an imide bond and an amino group (NH_3^+ deformation vibration of an amino acid). The HF treatment suggested the possibility that the amino group was linked with silicates. Two bands at 1090 and 470 cm^{-1} could be attributed to the Si-O-Si bond. The characteristic band (amide II band) of a peptide bond at 1550 cm^{-1} was absent.

The microspheres were dissolved with 1% SDS-8 M urea or 0.5% hydrofluoric acids. The microspheres completely disappeared on treatment with 1% SDS-8 M urea at 100°C

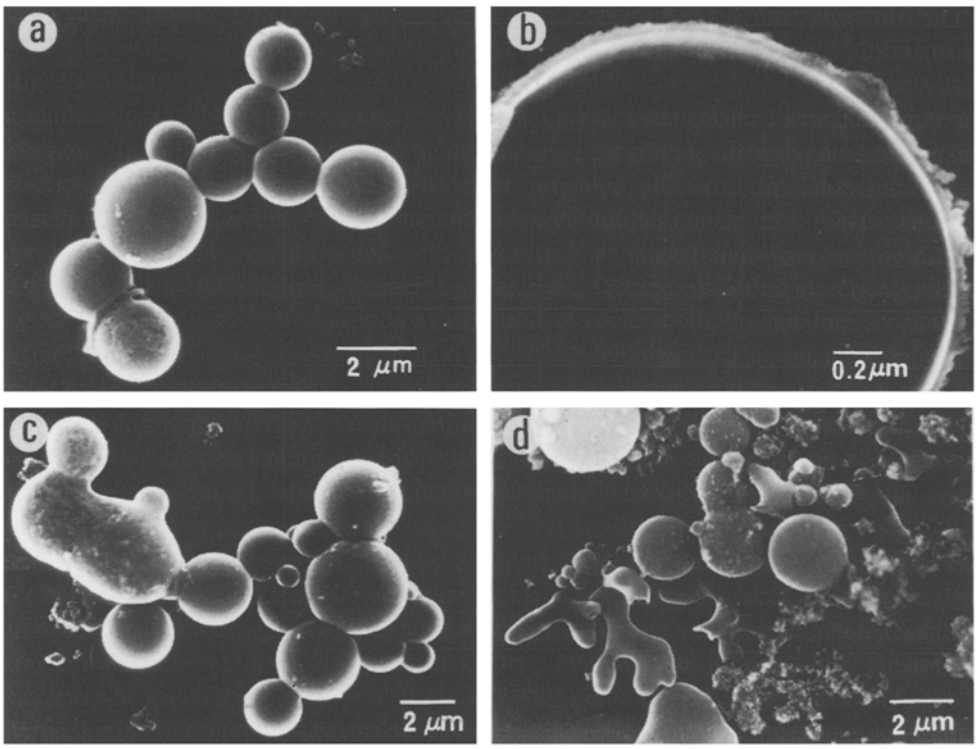

Fig. 4. Formation of microspheres in a reaction of mixture containing glycine, alanine, valine, and aspartic acid at 250°C(a), 300°C(c), and 350°C(d) under a hydrostatic pressure of 134 atm, as well as the interior structure of the microspheres produced at 250°C(b). A reaction mixture (15 ml) containing 0.3 M glycine, 0.1 M L-alanine, 0.3 M L-valine, 0.1 M L-aspartic acid, and 0.1 M $KHCO_3$-NaH_2PO_4 buffer (pH 7.2) was put into a pyrex tube (20 x 105 mm), which was capped and placed in a stainless steel autoclave (70 ml) which was encased in an aluminum heating block. The autoclave was heated at 200°C, 250°C, 300°C, or 350°C for 6 hours. The resulting solution was centrifuged and the precipitate was placed on a clean glass coverslip. The dried specimens were examined under a scanning electron microscope (JEM 100 CX-ASID). In order to observe the interior of the microsphere, freeze dried specimens were cracked using two pieces of adhesive vinyl tape, and then examined under the microscope.

for 10 min or 0.5% hydrofluoric acid at room temperature for 10 min. Polyacrylamide gel electrophoresis of the 1% SDS-8 M urea-solubilized components of the microspheres gave, after silver staining, a broad band with molecular weights of 1,000-2,000 daltons (Fig. 5a). The microspheres were partially hydrolyzed with 6 N HCl at 110°C for 72 hours. After the hydrolysis, 10% of the total amide bonds were cleaved and silica was found in the hydrolyzate. These results suggest that the microspheres are made of peptide-like polymers with the following characteristics: 1) resistance to acid hydrolysis, 2) silicon bonds are included, and 3) molecular weights of 1,000-2,000.

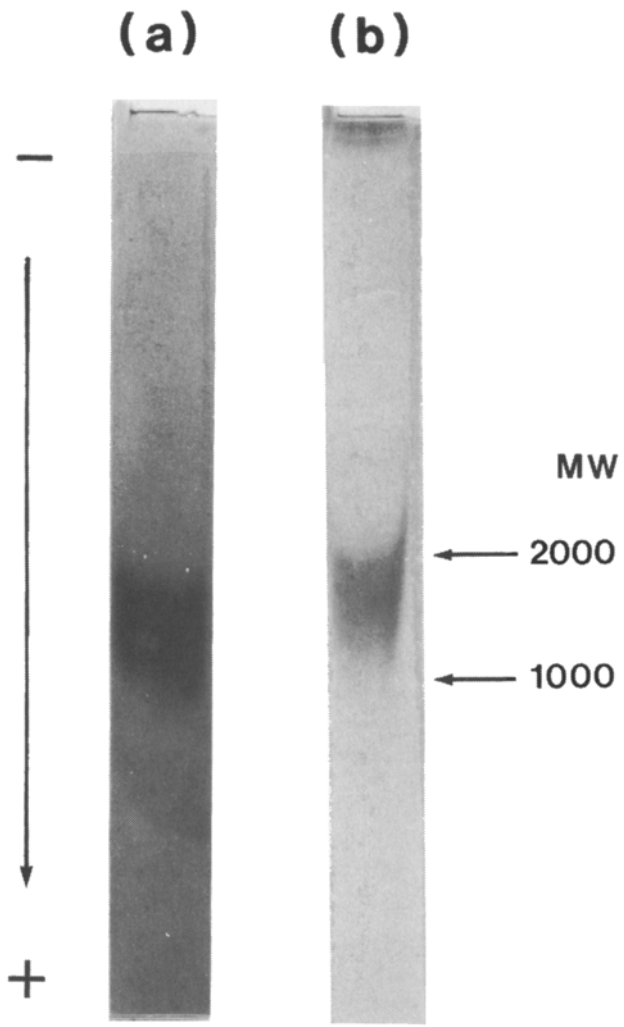

Fig. 5. Polyacrylamide gel electrophoresis of thermostable microspheres and supernatants of reaction mixture. (a) 1% SDS-8 M urea-solubilized components of microspheres produced at 250°C; (b) supernatants of reaction mixture heated at 250°C.

Peptide-like polymers were also present in the supernatant of the reaction mixture, and were found to remain on an Amicon YM-2 membrane after ultrafiltration. Polyacrylamide gel electrophoresis of peptide-like polymers in the supernatant gave, after silver staining, a broad band with molecular weights of 1,000-2,000 daltons (Fig. 5b). The electrophoretic pattern was very similar to that of the 1% SDS-8 M urea-solubilized component of the microspheres. The IR spectrum of the peptide-like polymers obtained

from the supernatant showed characteristic bands at 1670 (broad) and 1570 cm^{-1} which can be attributed to the imide and peptide bonds, respectively. They were partially hydrolyzed with 6 N HCl at 110°C for 72 hours. After such acid hydrolysis, 50% of the total amide bonds were cleaved and silica detected.

^1H NMR in D_2O of the peptide-like polymers found in the supernatant showed a signal at 0.82-1.10 ppm that suggested the presence of valine residues. Similarly, β-CH_3 of alanine residues, α-CH_2 of aspartic acids residues, and β-CH_2 of glycine residues appeared at 1.44-1.52 ppm, 2.42-2.98 ppm , and 3.58-2.62 ppm, respectively. The intensity of signals from valine residues was 4-fold that for glycine and that for alanine. ^1H NMR in DMSO-d_6 showed a signal at 8.07 ppm that suggested the presence of peptide bonds. Signals on ^{13}C NMR in D_2O at 18.5-19.3 ppm, 19.4-22.2 ppm., 44.7-46.1 ppm, and 52.7-56.9 ppm were assigned to β-CH_3 of alanine residues, γ-CH_3 of valine residues, α-CH_2 of glycine residues, and α-CH of aspartic acid residues, respectively. Similarly, the resonances at 173.7-179.2 ppm were assigned to the carbon of amide bonds, and the resonances at 183.7-188.3 ppm to the carboxyl group. These NMR studies showed that the peptide-like polymers contain valine, alanine, glycine, and aspartic acid residues which are linked by imide bonds and peptide bonds. Judging from the intensity of the signals, the content of valine was 4-fold that of any other amino acid.

Peptide-like polymers were also present in the supernatant of the reaction mixture. Fig. 6 shows gel chromatograms of the products of several reaction mixtures heated at different temperatures. The reaction mixture at 200°C gave a single peak with a molecular weight of 1,100 daltons. This peak can barely be detected at 100°C, increases at 150°C and is the sole product at 200°C (Fig. 6a). At 250°C several peaks with molecular weights up to 4,000 daltons appear in the chromatogram (Fig. 6b). A gel chromatogram of the product of the reaction mixture heated at 250°C in a stainless steel vessel was much the same as that of the reaction mixture heated at 250°C in a glass tube. At 300°C these peaks disappeared and a peak with a large molecular weight and a peak with a small molecular weight survived. (Fig. 6c). Judging from the fact that polyacrylamide gel electrophoresis of the large molecular weight peak gave a broad band with a molecular weight of 1000-2000 daltons (Fig. 5), this large molecular weight peak seems to be an aggregated form of peptide-like polymers. Apparently at 250°C and 300°C aggregation and degradation of the peptide-like polymers occurs simultaneously. At 350°C these peaks decreased considerably (Fig. 6d). As in the results discussed above, the NMR spectrum of the peptide-like polymers showed a high content of valine. The resulting peptide-like polymers were resistant to acid hydrolysis. The fraction of the polymers susceptible to hydrolysis decreased with a rise in the temperature of hydrolysis.

Based upon the physical data described above, a possible chemical structure of the peptide-like polymers of microspheres is considered. They consist of imide bonds and amino acid residues having an abundance of valine. The presence of imide bonds results in the branched structure of the polymer. Some amino groups near the branching point are linked by an aminosilyl (N-Si) bond to silica. The silica may contribute to the hy-

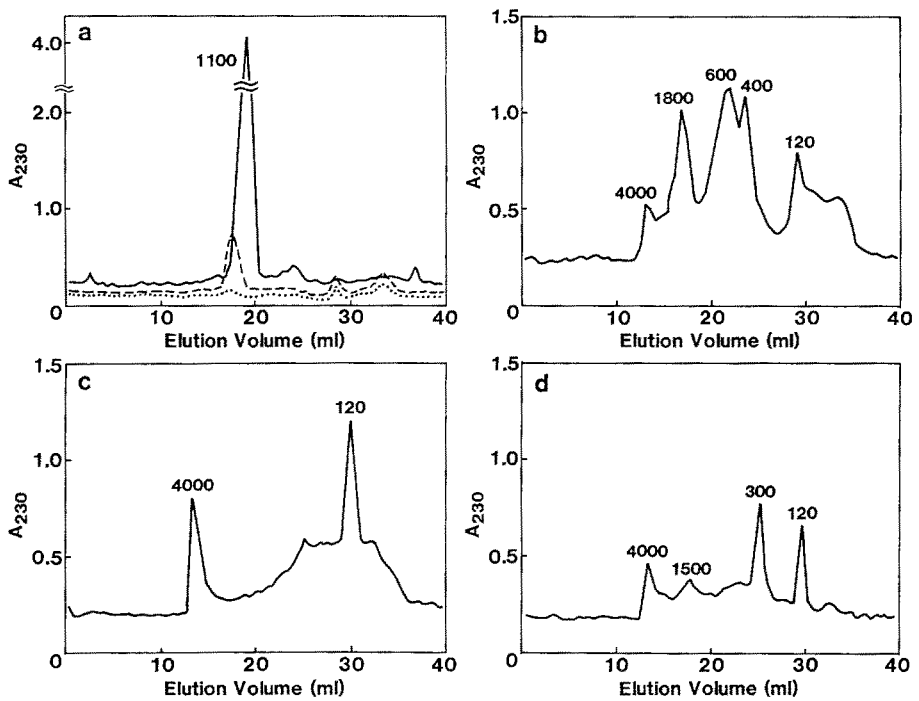

Fig. 6. Bio-Gel P-4 chromatography of supernatants of a reaction mixtures heated at different temperatures. (a) Supernatants of reaction mixtures heated at 100°C (...), 150°C(----), and 200°C(——) for 6 hours; (b), (c), and (d), supernatants of reaction mixtures heated at 250°C, 300°C and 350°C for 6 hours, respectively. Numerical values over peaks represent molecular weight of the peaks.

drophilic moiety of the polymers. The peptide-like polymers in the supernatant may be precursors of the peptide-like polymers found in the microspheres.

Concerning the stability of the aminosilyl bond, there must be some protective mechanisms. A peptide bond between two valines was very resistant to acid hydrolysis. For example, only 5% of the total peptide bonds of polyvaline were hydrolyzed with 6 N HCl at 110°C for 72 hours: only with trifluoroacetic acid-12 N HCl (1:1) at 175°C for 24 hours was polyvaline completely hydrolyzed. Thus, the aminosilyl bond may be protected by the hydrophobic moiety of valine residues from attack by water.

It may be that at lower temperatures of the reaction, polypeptides are produced that are rich in glycine, alanine, valine, and aspartic acid. (Yanagawa et al., 1990; Ito *et al.*, 1990). Peptide bonds composed of glycine, alanine, and aspartic acid may be preferentially cleaved because of their relative instability at 250°C as compared to peptide bonds between valine residues which can withstand hydrolysis at high temperature. Thus, the content of valine increases and peptide-like polymers having a preponderance of valine survive.

4. Implications for Hydrothermal Synthesis of Organic Compounds and Thermophilic Life

The experimental results summarized above indicate that abiotic synthesis of organic compounds may be possible in submarine hydrothermal systems. In addition, these results show that polymerization reactions may occur among organic compounds in hydrothermal fluids. These results are consistent with theoretical calculations which show enhanced stability of peptide bonds with increasing temperature in aqueous solution (Shock, 1992a). Further experiments on systems in which the pH, oxidation state and activities of inorganic ions are buffered to values consistent with observations of submarine hydrothermal systems will help clarify the extent to which the present results reflect what can happen in natural systems. One reason why these experiments demonstrate amino acid production rather than high temperature destruction documented by others (White, 1984; Miller and Bada, 1988; see above) may be the capacity of the high concentrations of transition metals in the solutions to constrain the oxidation state to fairly reduced levels.

These experiments may also have implications for life at high temperatures. Numerous studies have shown that extremely thermophilic organisms are found in submarine springs at temperatures of at least 110°C (Stetter, 1982; Jones *et al.*, 1983, 1989; Fiala and Stetter, 1986; Stetter *et al.*, 1983; Jannasch *et al.*, 1988; Zhao *et al.*, 1988; Huber *et al.*, 1989; Zillig *et al.*, 1990). Although some investigators argue against the possibility of life at temperature in excess of 200°C (Trent *et al.*, 1984; White, 1984; Miller and Bada, 1988, 1991a,b) recent results from submarine hydrothermal vents along the Endeavor Ridge show that particulate DNA is recovered from the fluids and survives incubation for several hours at vent temperature (Deming, 1991).

These results may indicate that life exists in present-day submarine hydrothermal systems at temperatures exceeding those for which bacteria have so far been cultured. Thermophilic bacteria capable of growing in hydrothermal environments are expected to have special mechanisms for adaptation to these environments (Baross, 1991). The results summarized above indicate that extremely thermophilic cellular structures may be possible if they are somehow analogous to the microspheres produced from amino acids at 250°C.

Acknowledgements

We thank Dr. T. Onoda, Dr. K. Wada, Dr. M. Dokiya, and Y. Kasori for performing reactions at high temperatures, Dr. T. Hayase and H. Kosono for obtaining the NMR spectra, S. Kondo for obtaining the electron micrographs. We express our gratitude to Prof. E. L. Shock for helpful discussion and providing some text. This work was partly supported by a Grant-in-Aid (No. 63540445) from the Ministry of Education, Science and Culture, Japan.