# In Situ Measurements of Chemical Distributions in a Deep-Sea Hydrothermal Vent Field

KENNETH S. JOHNSON, CARL L. BEEHLER,
CAROLE M. SAKAMOTO-ARNOLD, JAMES J. CHILDRESS

**Large changes in the concentration of sulfide around a hydrothermal vent in the Galápagos Rift provide direct evidence for the consumption of sulfide by the organisms of the vent community. These changes were detected with a new chemical analyzer capable of measuring silicate, sulfide, oxygen, and temperature on the sea floor at depths of 2500 meters. More than 10,000 measurements showed systematic variations in the sulfide and oxygen concentrations due to biogenic oxidation of sulfide in the hydrothermal solutions. Silicate concentration was highly correlated with temperature, but different trends were observed at different locations.**

EXTENSIVE COMMUNITIES OF ORGANISMS have been found associated with submarine springs along mid-ocean ridges (*1, 2*), subduction zones (*3*), the Florida Escarpment (*4*), and the continental slope off Louisiana (*5*). These dense communities are remarkable because the deep sea is usually characterized by little available energy and a low density of organisms. However, vent organisms can metabolize reduced compounds, particularly sulfide (*6*), that are present in high concentrations in the hydrothermal solutions (*1, 7, 8*). The carbon isotopic composition of the animals also indicates that they utilize a nonphotosynthetic carbon source (*9*). Despite the importance of these reduced chemicals, there has been no extensive study of their distributions around the animals in these communities. Such a study is critical to our understanding of the community structure and metabolism.

We have developed a submersible chemical analyzer (Scanner) that is capable of continuous in situ chemical analysis in the deep sea. The Scanner, mounted on the submersible *Alvin*, was used in the Rose Garden hydrothermal vent system of the Galápagos Rift at a depth of 2500 m (*10*). Over 10,000 chemical measurements were made in situ during this expedition, the first such measurements in the deep sea other than electrochemical determinations of *p*H, oxygen concentration, and conductivity. The measurements showed large changes in the composition of the venting water over short distances, an indication that the hydrothermal vent community influences the composition of the water.

The Scanner was designed to perform two colorimetric chemical analyses simultaneously. Dissolved silicate and sulfide were analyzed on the Galápagos 1985 expedition. Sulfide was measured because it is believed to be the primary energy source for the major sessile animals (*6*). Silicate was determined because it is a good tracer of the fraction of vent water present in a sample (*1, 7*). Dissolved oxygen, which is necessary for the oxidation of sulfide by chemoautotrophs, was also monitored in the Scanner with an amperometric oxygen electrode. Temperature was recorded at the sample inlet with a thermistor.

Chemical analyses were performed with a flow injection analysis (FIA) technique (*11*) modified for continuous analyses (*12*). Detector outputs were read every 10 seconds and recorded digitally. Silicate and sulfide standard solutions were analyzed in situ at 40-minute intervals to calibrate the Scanner. Two standard deviations for these measurements in ambient bottom water (2.07°C) were ±0.1 μ*M* for sulfide, ±2 μ*M* for silicate, ±3 μ*M* for oxygen, and ±0.01°C for temperature.

The Scanner was mounted on the outside of the submersible *Alvin*. The microprocessor control system of the Scanner was protected by a pressure housing; the other components (pump, valves, detectors, and manifold) were pressure-tolerant. The sample inlet was at the end of a probe attached to one manipulator so that scientists could precisely position the inlet. The Scanner output was transmitted into the submersible and displayed graphically in real time to aid the observers with sampling.

Chemical distributions were surveyed around three separate, but adjacent (within 30 m of one another) clumps of vent animals on *Alvin* dive 1528 in the Rose Garden vent field. The hydrothermal vent mussel *Bathymodiolus thermophilus* was the major inhabitant in the three clumps (labeled locations A, B, and C). Figure 1 shows the results from location C. The measurements were made by moving the probe from ambient (2°C) bottom water as far as possible into the clump of animals at an angle of about 45°. The probe was moved a distance of about 50 cm during the period shown in Fig. 1.

Our in situ measurements reflect the trends expected from earlier studies; that is, silicate and sulfide increased with temperature, whereas oxygen decreased (*1, 7, 13*). However, the Scanner data provide far greater detail than past studies. The 180 determinations shown in the 30-minute record of Fig. 1 exceed the total number of all earlier samples from the Galápagos Rift hydrothermal vent system.

Silicate was linearly related to temperature

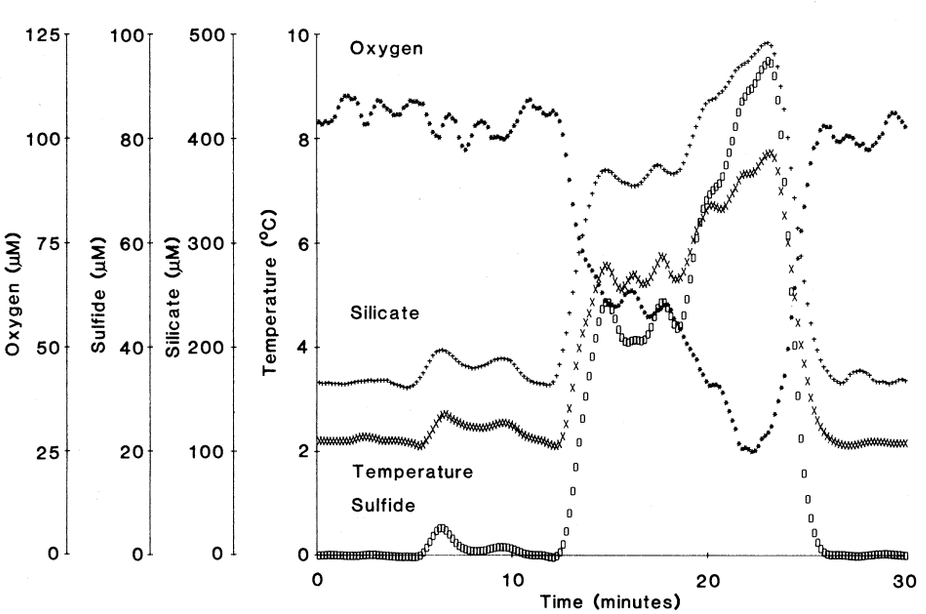


Fig. 1. Temperatures and silicate, sulfide, and oxygen concentrations plotted versus time at location C in the Rose Garden vent site on *Alvin* dive 1528. We smoothed all data, using an 11-point, quadratic Savitzky-Golay convolution (*21*). The temperature and oxygen data were also dampened with a numerical filter to reduce their response rates to that of the silicate and sulfide channels.

Marine Science Institute, University of California, Santa Barbara 93106.

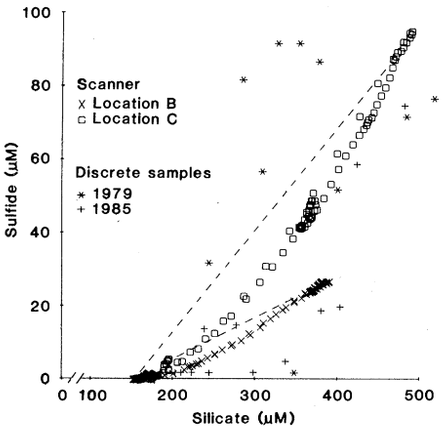


Fig. 2. Sulfide concentration plotted versus silicate concentration at locations B and C of the Rose Garden vent site on *Alvin* dive 1528. Data determined at the Rose Garden from discrete samples taken in 1979 (*22*) are also plotted. The straight lines connecting the extremes of the in situ data at each location indicate the trends on which the data would lie if only mixing occurred when the end members were combined.

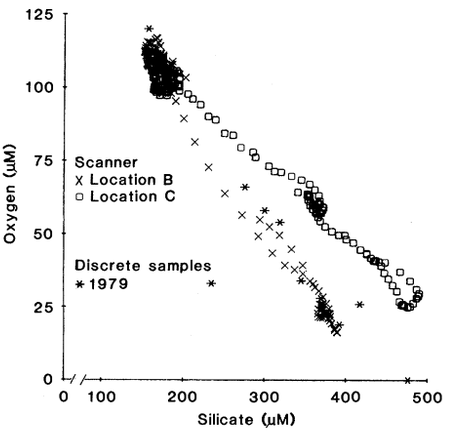


Fig. 3. Oxygen concentration plotted versus silicate concentration at locations B and C in the Rose Garden vent site on *Alvin* dive 1528. Measurements on discrete samples collected at the Rose Garden in 1977 (*22*) are shown for comparison.

at each location (Table 1). The slopes of the least-squares lines ($b$) at locations A and C were similar to the earlier value measured (0.0160) in the Galápagos hydrothermal vent system (*13*). However, the slope at location B was significantly ($P < 0.01$, $t$ test) greater than 0.160 by 40 ± 2 percent.

It is highly unlikely that the different slopes of the temperature and the silicate data were caused by dissimilar compositions of the hydrothermal source solution, in view of the close proximity of the three locations. The different slopes imply that silicate or temperature or both are not conservative when the hydrothermal solution mixes with seawater. Silicate can be removed by precipitation in the subsurface to form the stockwork vein quartz deposits common in ophiolites (*14*), or it can precipitate in hydrothermal deposits on the sea floor (*15*). Lower temperatures may also result from a greater conductive heat loss to the surrounding rock. Either of these processes could be responsible for the different slopes of the temperature and the silicate data.

The linear relation between silicate and temperature in our data suggests that these substances are conservative at temperatures below 10°C. Both precipitation of silicate and conductive heat loss would tend to be greater at high temperatures. They apparently occur primarily in the subsurface zone.

The results of our silicate and sulfide analyses at locations B and C are plotted in Fig. 2. Location A was similar to location C. Conservative mixing between hydrothermal fluid and seawater would produce a straight-line relation between these chemicals (Fig. 2). Only the removal of sulfide from the mixture of hydrothermal solution and seawater within the mussel clumps could have produced the significant curvature in the sulfide-silicate data ($P < 0.01$, $F$ test) at all three locations. Removal at location C was greatest near 35 μ$M$ sulfide, where the deviation from a straight line connecting the extremes of the data is the largest.

There was a close link between the removal of both sulfide and oxygen at the sites we studied. For example, both sulfide and oxygen concentrations at location B were considerably lower than the values measured at equivalent silicate concentrations (or temperatures) at location C (Figs. 2 and 3) and location A. This trend was found in all of our data: locations with the most sulfide removed also had the most oxygen removed. The corresponding decreases in both sulfide and oxygen indicated that the sulfide was removed by oxidation rather than by precipitation as a metal sulfide.

Attached macro- and microorganisms were probably responsible for the removal of most of the sulfide and oxygen. Spontaneous oxidation and consumption by suspended bacteria appeared to be of less importance in this system. The in situ removal rate of sulfide was conservatively estimated to be on the order of 1 μmol/liter per minutes (*16*). Discrete water samples collected at the vents would continue to lose sulfide at this rate if oxidation were due to suspended bacteria entrained in the sample or to inorganic reaction. Little or no sulfide would then have remained in the water samples after the 5- to 10-hour period between collection and analysis (much of which was at in situ conditions) in this case. However, in situ measurements made with the Scanner were in reasonable agreement with measurements on discrete water samples made in 1979 and 1985 (Fig. 2). The very low sulfide concentrations in some of the discrete samples are in agreement with Scanner data from other dives. These values reflect the almost complete removal of sulfide from the vent water in areas with the highest animal population density. The sulfide oxidation rate in the water samples must have been much lower than the rate measured in situ around the animals.

The vent mussels, which dominated the population at each of the locations, are able to metabolize sulfide (*17*). The apparent absence of detectable concentrations of sulfide in discrete samples taken around mussels in the Galápagos Rift during 1979 has led to the suggestion that particulate organic carbon is an important energy source for these animals (*18*). However, the communities that we studied must have been actively consuming sulfide to produce the large deficits that were observed. The lesser removal of sulfide at locations A and C, relative to that at location B, may reflect differences in the density of organisms or in the rate of venting at the three sites.

The oxygen-silicate data appear more linear than the sulfide-silicate data (Fig. 3), although greater scatter in the oxygen data

Table 1. Parameters of the equation $T = a + b$[Si], where $T$ is temperature (in degrees celsius) and [Si] is the silicate concentration (in micromoles per liter) at locations A, B, and C in the Rose Garden vent site studied on *Alvin* dive 1528. Results from the Galápagos Rift in 1977 (*7*) are given for comparison. Equations were fitted to our data by an autoregression procedure (*20*) to correct for autocorrelation introduced by smear in the pumping system. Errors are 1 SD. Temperature data were dampened numerically to reduce the apparent response time of the thermistor to that of the silicate channel. RMSE is the root-mean-square error of the temperature residuals ($n - 2$ df) from the fitted line.

| Location | $a$ | $b$ | $n$ | RMSE |
|---|---|---|---|---|
| A | −0.46 ± 0.17 | 0.0151 ± 0.0009 | 180 | 0.205 |
| B | −1.81 ± 0.07 | 0.0237 ± 0.0003 | 180 | 0.154 |
| C | −0.60 ± 0.06 | 0.0165 ± 0.0002 | 180 | 0.123 |
| | | *Results from 1977 (7)* | | |
| | −0.15 ± 0.22 | 0.0160 ± 0.0005 | 49 | 0.568 |

makes curvature harder to detect. If oxygen was consumed primarily from the oxygenated bottom water, then the oxygen could have been resupplied from the surroundings at a high rate. This would prevent any large curvature from appearing and oxygen would seem to behave conservatively. Oxygen uptake by the hydrothermal vent clam *Calyptogena* appears to occur at near ambient conditions, whereas sulfide is consumed in warmer water with higher sulfide concentrations (*19*).

There was 27 μ*M* less sulfide and 37 μ*M* less oxygen at location B than at location C at a silicate concentration of 400 μ*M*. The extra sulfide and oxygen consumed at location B was removed in a ratio of 0.73 to 1. It is difficult to determine the oxidation product of sulfide from these results, however, because community oxygen demand will consume oxygen without sulfide. Anaerobic sulfide oxidation is also possible.

Our in situ measurements demonstrate large variability in sulfide and silicate concentrations in the vicinity of the animals of this hydrothermal vent community. Consumption of sulfide by the vent animals was also evident. Direct calculations of the community metabolism will be possible when measurements of flow rates around these animals become available.


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# Endonucleolytic Activity That Cleaves Immunoglobulin Recombination Sequences


THOMAS J. HOPE, RENATO J. AGUILERA, MARK E. MINIE, HITOSHI SAKANO



**An endonucleolytic activity has been identified in nuclear extracts of chick embryo bursa and mouse fetal liver cells. The activity introduces a double-strand cut in the vicinity of the recombination site of immunoglobulin joining gene segments. The cleavage occurs at the dinucleotide pair $\frac{TG}{AC}$. This activity is a good candidate for the putative endonuclease involved in recombination of the immunoglobulin variable, diversity, and joining regions. It is distinct from the endonuclease activities previously reported by others.**


IMMUNOGLOBULIN (IG) VARIABLE REgion genes are generated by site-specific DNA recombinations during the differentiation of B lymphocytes (*1*). In the light chain genes, the recombination takes place between two DNA segments, V and J (*2–5*). In the heavy chain genes, an additional DNA segment D (diversity) is involved (*6–8*); thus, two recombination events, V-D and D-J joinings are necessary to generate a complete heavy chain gene.

Nucleotide sequence analysis of the germline DNA segments revealed that two consensus sequences, CACTGTG and GGTTTTTGT, are always found at the recombination site, and the spacer separating the two sequences is either 12 or 23 base pairs (bp) long (*3, 6–8*). DNA recombination takes place between two pairs of palindromic conserved sequences, with one pair being separated by a 12-bp spacer and the other by a 23-bp spacer (12–23 bp spacer rule) (*6, 7*).

In order to better understand the molecular mechanism of Ig gene recombination, we have attempted to identify a recombination enzyme which mediates V-(D)-J joining. Our studies are based on the assumption that three main activities should be involved in Ig gene recombination: a DNA binding activity, an endonucleolytic activity, and a ligase activity. The binding activity could bring the two recombination sites together into the proper orientation, satisfying the 12–23 bp spacer rule. The endonucleolytic activity is then required to cut or nick the germline DNA around the recombination site. Finally, the ligase activity must join the two Ig sequences covalently.

We first attempted to identify a specific endonucleolytic activity that cleaves Ig gene sequences at or near the recombination site (*9*). We used mouse fetal liver and chick embryo bursa as enzyme sources because B-cell progenitors are assumed to first appear in these tissues during embryonic development (*10, 11*). We prepared nuclear extracts from these tissues (*12*), and fractionated them by column chromatography. An endonucleolytic activity that cleaves in the Ig joining segment region was identified by a Southern blot hybridization assay. To ascertain where the joining region cleavage occurs we used a DNA sequencing gel system (*13*) to analyze the cleavage products with chemically cleaved DNA as a size marker. We now report that mouse fetal liver nuclei contain an endonucleolytic activity that cleaves mouse $J_{\lambda 1}$ (λ, lambda chain) DNA between the conserved heptamer and the $J_{\lambda 1}$ coding sequence.


Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.