Chemical speciation drives hydrothermal vent ecology

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The physiology and biochemistry of many taxa inhabiting deep-sea hydrothermal vents have been elucidated[1–4]; however, the physicochemical factors controlling the distribution of these organisms at a given vent site remain an enigma after 20 years of research[5–11]. The chemical speciation of particular elements has been suggested as key to controlling biological community structure in these extreme aquatic environments[7,11,12]. Implementation of electrochemical technology[13,14] has allowed us to make *in situ* measurements of chemical speciation at vents located at the East Pacific Rise (9° 50′ N) and on a scale relevant to the biology. Here we report that significant differences in oxygen, iron and sulphur speciation strongly correlate with the distribution of specific taxa in different microhabitats. In higher temperature (> 30 °C) microhabitats, the appreciable formation of soluble iron-sulphide molecular clusters markedly reduces the availability of free H_2S/HS^- to vent (micro)organisms, thus controlling the available habitat.

To determine the availability of biologically relevant chemical compounds in mixing zones from diffuse flow venting areas, *in situ* chemical analyses are essential[7,11,12]. Earlier *in situ* measurements at hydrothermal vents used flow injection analysis to measure acid-volatile sulphide (AVS = Σ[free H_2S/HS^- plus FeS])[11,15], but this technique does not permit identification of individual sulphur components. At a given vent location, AVS generally increases with temperature, as expected from conservative mixing; however, no clear chemical pattern that can explain the different environmental niches occupied by different vent organisms has been discerned[11]. As iron- and sulphide-rich vent fluids meet ambient seawater, dissolved iron reacts with sulphide to form solid FeS ('black smoke')[16], which reduces the availability of free H_2S to organisms. Studies[16–18] have shown that dissolved molecular clusters of FeS (FeS (aq)) can be discriminated from free H_2S/HS^- and polysulphides by voltammetry. Here we have used an *in situ* voltammetric sensor package for real-time measurements at deep-sea hydrothermal vents.

Surveys of ambient deep-sea water and vent end-member fluids showed the expected chemistry. Linear sweep voltammograms (LSVs) from ambient seawater revealed only the presence of O_2 (Fig. 1a). Conversely, cyclic voltammograms, taken with the electrodes placed 0.5 m (25 °C) above a black smoker, showed only the presence of free H_2S/HS^- and FeS (aq) (Fig. 1b). These results provided the framework for comparative studies of the habitats for different organisms: the tubeworm *Riftia pachyptila* and the polychaete *Alvinella pompejana*. The tubeworm resides in lower temperature fluids, issuing through seafloor openings or from the base of chimneys (< 25 °C), and harbours bacterial endosymbionts[1,2], which produce organic carbon for the host by the bacterially mediated chemosynthetic reaction of H_2S/HS^- with CO_2. In contrast, *A. pompejana*, which forms tube dwelling colonies on the sides of active sulphide chimneys (> 25 °C), does not rely on chemosynthetic endosymbiots but directly ingests bacteria in, on and outside its tubes[19].

In a community of living *R. pachyptila*, electrodes were placed near their plumes, where acquisition of H_2S/HS^-, CO_2, and O_2 occurs for chemoautotrophy. Only H_2S/HS^- and O_2 were measured

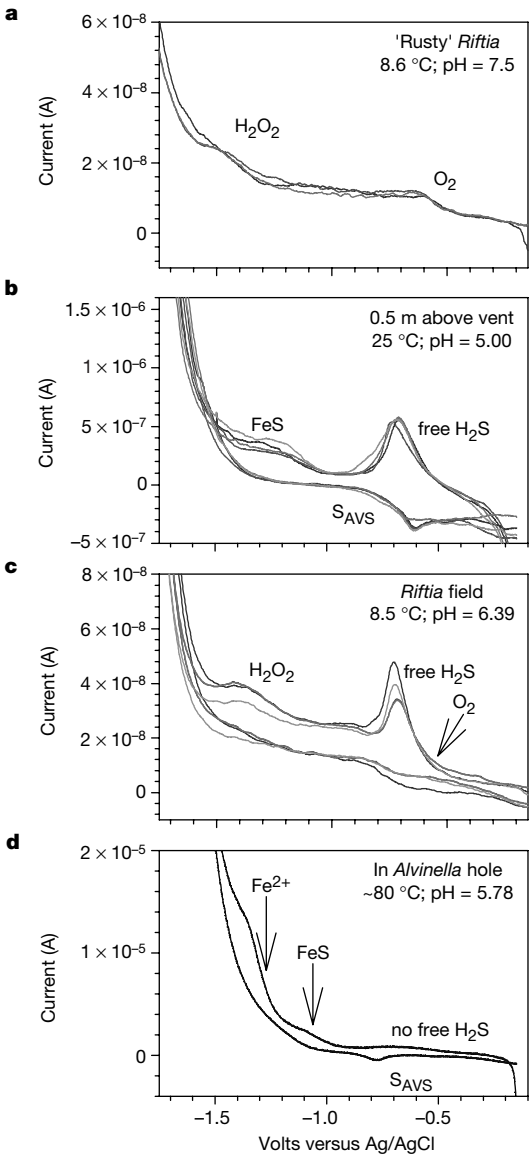

Figure 1 Representative *in situ* voltammetry data. **a**, LSV scans at dead *Riftia* sites or where vent biota do not exist. O_2 reacts to form H_2O_2 at a half-wave potential ($E_{1/2}$) of –0.5 V, and the resulting H_2O_2 reduces to water at –1.30 V. H_2O_2 overlaps with the Fe^{2+} signal so traces of Fe^{2+} cannot be discerned. **b**, Cyclic voltammogram scans taken 0.5 m above a vent orifice showing free H_2S/HS^- and FeS (aq). Free H_2S/HS^- reacts to form a HgS film at positive potentials and is reduced during the negative scan to release sulphide at $E_{1/2}$ = –0.65 V. FeS (aq) gives an irreversible signal ($E_{1/2}$ = –1.12 V) owing to reduction of Fe^{2+} in FeS (aq) to Fe(Hg) which decomposes FeS (aq) (ref. 17). On scan reversal, the negative current signal ($E_{1/2}$ = –0.62 V) is from all sulphide (AVS = free H_2S/HS^- and sulphide released from FeS (aq)) reacting with Hg to reform HgS. **c**, Cyclic voltammogram scans from *Riftia* sites showing only O_2 and free H_2S/HS^-; the AVS signal is small when only H_2S/HS^- is present. **d**, Cyclic voltammogram scan (200 mV s^{-1}) taken at 80 ± 20 °C from within an *Alvinella* tube shows Fe^{2+} ($E_{1/2}$ = –1.30 V) and FeS (aq) ($E_{1/2}$ = –1.05 V) as the main chemical species. The negative current signal (–0.76 V) results only from the breakdown of FeS (aq). The current axis is much higher because it is a function of temperature.

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by voltammetry (Fig. 1c), consistent with previous observations[11]. Another survey from an assemblage of dead *R. pachyptila* showed that surrounding waters contained only O_2 (Fig. 1a), even though the waters (8.5 °C) were above ambient deep-sea temperatures (2 °C). The tubes were also encrusted with orange-brown solids containing Fe(III). Absence of free H_2S/HS^-, caused by a change in chemistry of emanating vent fluids, indicates that sulphide chemosynthesis cannot occur and support a thriving *R. pachyptila* community.

Chemical speciation of *A. pompejana* habitats was significantly different from live and dead *R. pachyptila* areas. The electrodes, placed directly inside occupied *A. pompejana* tubes, revealed large signals (Fig. 1d) due to FeS (aq) and Fe^{2+}. Free H_2S/HS^- was not detected, or only at trace levels, with no detectable O_2. Scans taken at tube openings and on the sulphide structure—the substrate for the *A. pompejana* colonies—showed similar iron and sulphur chemistry. These data indicate that chemical speciation is unique to the sulphide chimney structure and is not being modified by the *A. pompejana* colony. Free H_2S/HS^- was detected higher above the colony where the temperature approaches that of ambient seawater, suggesting that FeS dissociation occurred.

In addition, discrete samples[20] taken from 8 *R. pachyptila* and 11 *A. pompejana* locations (Fig. 2) were analysed aboard ship for AVS, pH and total iron (Fe_T) concentrations. AVS concentrations were at least five times higher at *A. pompejana* sites than at *R. pachyptila* sites, in accord with previous work[6,10]. H_2S/HS^- is the dominant sulphide phase in live *R. pachyptila* fields, and in six of the eight samples, free H_2S/HS^- ranged from 68 to 100% of the AVS. In contrast, FeS (aq) is the chief sulphide form in *A. pompejana* tubes and near their tube openings. Free H_2S/HS^- ranged from 0 to 39% of the total AVS in *A. pompejana* habitats, and its concentration is typically lower than that measured in *R. pachyptila* fields even though the total AVS is significantly higher.

At higher iron and AVS concentrations where *A. pompejana* resides (Fig. 3), formation of solid FeS and FeS (aq) is enhanced because of higher temperatures[16,21]. This reduces the free H_2S concentration while lowering pH to about 5 (eq. 1). This reaction is supported by the significant correlation of Fe_T versus AVS for *A. pompejana* samples ($r^2 = 0.574$; slope $Fe_T/AVS = 1.52$). There are also significant inverse correlations between Fe_T or AVS versus pH for the *A. pompejana* samples from the different sites (Fig. 3). We propose that FeS (aq) molecular cluster formation is the primary mechanism controlling free sulphide detoxification for *A. pompejana* in vent waters.

$$Fe^{2+} + H_2S \rightleftharpoons FeS(aq) + 2H^+ \quad (1)$$

In contrast, *R. pachyptila* resides in lower temperature (< 25 °C) and higher pH (> 5.7) waters where there is no correlation of AVS versus pH (Fig. 3) and of Fe_T versus AVS ($r^2 = 0.019$). Thus, FeS formation is insignificant. In *R. pachyptila* fields, we did not observe a signal for FeS (aq), and free H_2S/HS^- is the dominant sulphur species. We detected trace amounts of Fe_T in three of the eight locations, and calculations indicated that the K_{sp} (solubility product constant) for FeS was not exceeded in *R. pachyptila* communities.

Unlike *A. pompejana*, *R. pachyptila* requires free sulphide so the bacteria endosymbionts can convert CO_2 to organic matter for translocation to their host. Research indicates that *R. pachyptila* transports and uses HS^- preferentially to H_2S (ref. 4). As the first acid dissociation constant (pK_a) of H_2S in seawater is 6.7 (ref. 22), at pH 5.7 only 10% of the free sulphide is HS^-. This suggests why *R. pachyptila* is observed in higher pH waters with low Fe_T and AVS concentrations, as compared with *A. pompejana*. Where the pH in diffuse flow waters is typically higher than 6.5 and iron is present in micromolar or lower amounts, as at Guaymas Basin in the Gulf of California, *R. pachyptila* is the dominant macrofaunal form and *A. pompejana* is not found. The presence of H_2S along with the two endosymbiont-containing vent bivalves (*Bathymodilus thermophilus* and *Calyptogena magnifica*), which also inhabit low-temperature diffuse flow areas, follows a similar pattern to that of *R. pachyptila*.

In situ electrochemical speciation and discrete sample measurements (temperature, pH, sulphide, Fe_T) provided unprecedented correlations with the distribution of certain hydrothermal vent

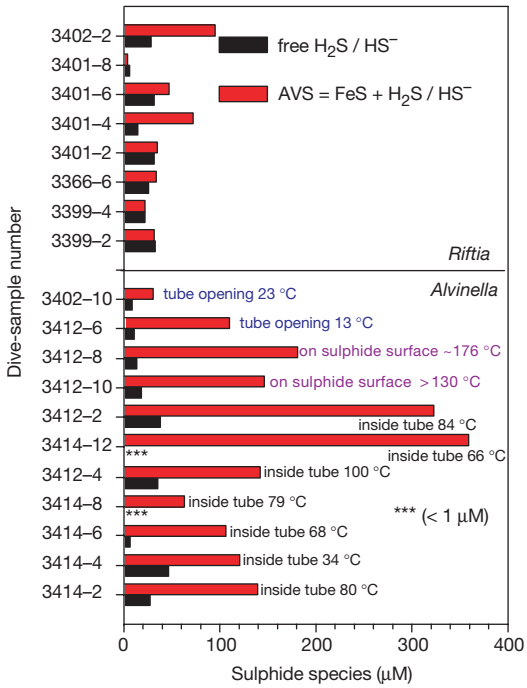

Figure 2 Histogram of free H_2S/HS^- and AVS data from discrete samples. For *Alvinella* samples at temperatures higher than 40 °C, temperature fluctuated rapidly during the *in situ* voltammetry scans, therefore free H_2S/HS^- data are from samples analysed on board ship. Figure 1d is from sample 3414-2 and shows no free H_2S/HS^-; thus, FeS dissociates to Fe^{2+} and free H_2S/HS^- on cooling and transport of samples to the surface. Free H_2S/HS^- data plotted for *Alvinella* samples are an overestimate of the actual free H_2S/HS^- that the organisms actually experience. For *Riftia* samples, both *in situ* and onboard voltammetry data give similar free H_2S/HS^- data.

macrofauna and evidence for the close association of vent community development with physiochemical conditions. *R. pachyptila* is found in modest lower temperature environments with low Fe_T, circumneutral pH and sufficient quantities of free sulphide to support robust bacterial endosymbiosis. The ability of *A. pompejana* to thrive in extreme microhabitats is made possible by the unique interaction of Fe^{2+} and free sulphide to form FeS species at high temperature, which lowers pH and detoxifies sulphide by rendering it biologically unavailable. Our *in situ* analyses have demonstrated the first real opportunity, at biologically relevant spatial scales, to show how differences in chemical speciation control the unique ecology of vent environments. The interplay of O_2, H_2S, Fe^{2+} and FeS (aq) in controlling biology in primordial environments on Earth could provide a paradigm for the detection of life on other planets. □

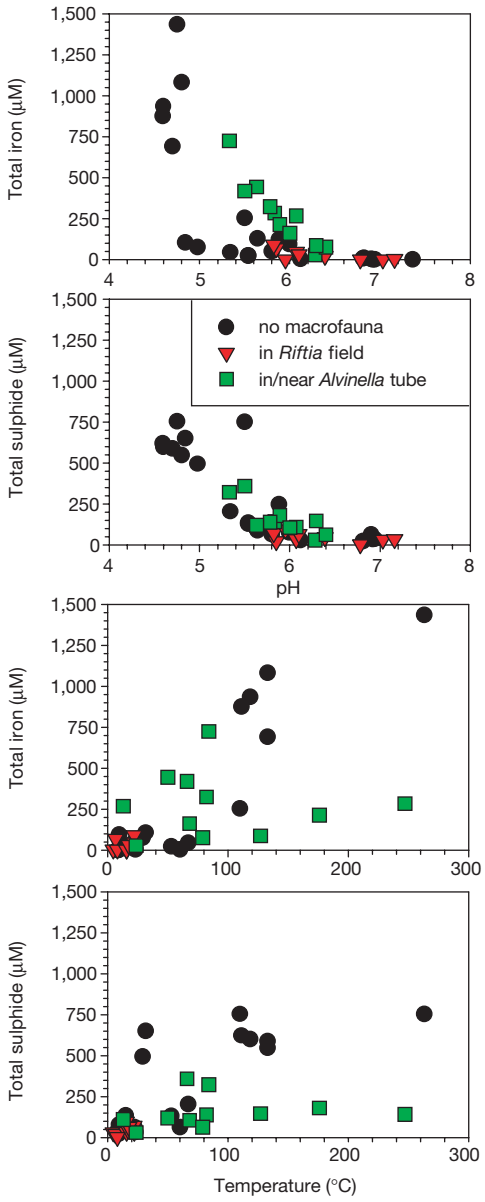

Figure 3 Fe/AVS versus pH/temperature plots from discrete samples. Circles, samples where no macrofauna exist; triangles, samples from *Riftia* sites; squares, samples from in or near *Alvinella* tubes. Correlations for *Alvinella*: Fe versus pH (r^2 = 0.880); Fe versus temperature (r^2 = 0.0003); AVS versus pH (r^2 = 0.647); AVS versus temperature (r^2 = 0.018). For *Riftia*: Fe versus pH (r^2 = 0.533); Fe versus temperature (r^2 = 0.156); AVS versus pH (r^2 = 0.280); AVS versus temperature (r^2 = 0.327).

Methods

Electrochemical equipment

The *in situ* analyser consists of a pressure case enclosing the voltammetry hardware linked to an IBM compatible computer. This computer communicates with another in the *Alvin* through a 15-m RS 232 cable and is controlled by an operator, who can reprogram waveforms to respond to the radically different environments found at vents. A separate 1-m cable was used to make connections with the working, counter, reference electrodes and the pressure housing. This cable had four inputs for working electrodes, which were controlled by a multiplexer, one input for the counter electrode and another for the reference electrode. Another input for grounding the reference electrode from the submersible ensured signal integrity. The working electrodes are solid-state gold-amalgam (Au/Hg), and were prepared in polyethyletherketone (PEEK) tubing[14]. The reference electrode was Ag wire oxidized in 3 M KCl to make an AgCl coating. This Ag/AgCl reference was used as a solid-state electrode as seawater has a consistent ionic strength of 0.7. Potentials for chemical species measured *in situ* and on board ship with the same reference electrode were comparable so no pressure effects were observed. Scan rates were 1,000 mV s^{-1} unless stated otherwise. Conditioning between scans was performed at –0.9 V, where none of the chemical species is electroactive[13,14]. Potential scan direction was from positive to negative for LSV. For cyclic voltammograms, the potential scan was positive to negative back to positive.

Analyses

The electrodes were mated with a thermocouple and syringe sampler ('sipper') to obtain discrete samples for shipboard analyses[20]. In some experiments, the electrodes, thermocouple and sipper tube were coupled as a sensor package for direct insertion into *Alvinella* tubes to analyse that chemical environment directly. We measured pH by potentiometry on these samples immediately after bringing them to the surface. Aliquots of each sample were preserved in basic zinc acetate solution for subsequent AVS analysis by square-wave voltammetry on a hanging-drop mercury electrode after a 3-M HCl acid leach to release H_2S, which was trapped in 1 M NaOH (ref. 23). This method gave identical results with the methylene blue method[15]. Fe_T was measured on subsamples stored in nitric acid[24]. We also measured unfiltered samples by cyclic voltammograms on board ship to confirm any chemical speciation changes.

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Perceiving visual expansion without optic flow

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When an observer moves forward in the environment, the image on his or her retina expands. The rate of this expansion conveys information about the observer's speed[1] and the time to collision[2–4]. Psychophysical[5–7]and physiological[8,9]studies have provided abundant evidence that these expansionary motions are processed by specialized mechanisms in mammalian visual systems. It is commonly assumed that the rate of expansion is estimated from the divergence of the optic-flow field (the two-dimensional field of local translational velocities)[10–14]. But this rate might also be estimated from changes in the size (or scale) of image features[15]. To determine whether human vision uses such scale-change information, we have synthesized stochastic texture stimuli in which the scale of image elements increases gradually over time, while the optic-flow pattern is random. Here we show, using these stimuli, that observers can estimate expansion rates from scale-change information alone, and that pure scale changes can produce motion after-effects. These two findings suggest that the visual system contains mechanisms that are explicitly sensitive to changes in scale.

To test the hypothesis that the rate of image expansion on the retina might be estimated from changes in scale (Fig. 1), we designed three types of stochastic expansion stimuli (Fig. 2a). We created a sequence of uncorrelated white-noise images, and convolved each one with a band-pass filter whose centre frequency decreases exponentially with time. The result is a sequence of random textures whose average spatial scale grows according to a fixed rate of expansion. Because the individual stimulus frames are uncorrelated, both the optic-flow field and its divergence are (on average) zero, and thus cannot be used to compute expansion rate. Nevertheless, most people who have viewed these stochastic stimuli report a strong perception of expansion, emanating from the point of fixation. This suggests that humans have mechanisms that are

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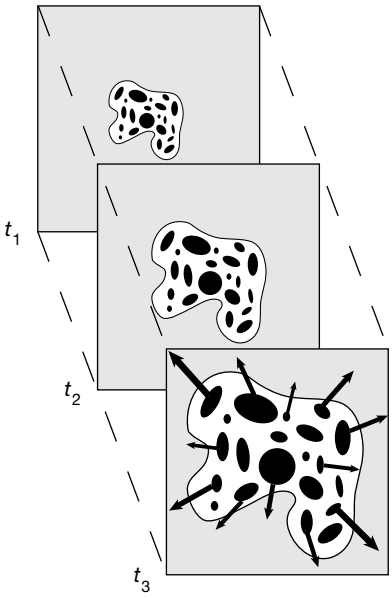

Figure 1 Illustration of visual input when an observer moves forward in the environment. Shown are three frames taken from a sequence of visual images (a movie). Superimposed on the last frame are example optic-flow vectors, indicating the local translational motion of selected points in the scene. The radiating pattern of these vectors indicates that the observer is moving forward. The divergence of this vector field provides one source of information about the rate of expansion. The change in scale of the objects or texture elements provides another source of information.

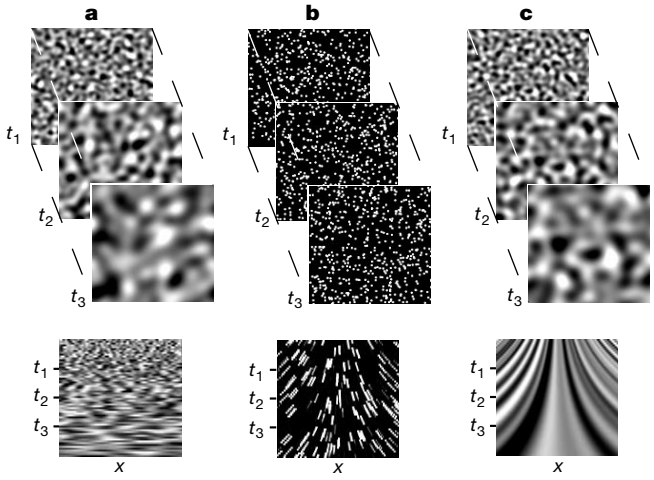

Figure 2 Illustration of the three types of stimuli used in our experiments. Top, example *x*–*y* frames of the stimulus movie; bottom, *x*–*t* cross-sections. **a**, Stochastic texture stimulus. Each frame contains a random texture synthesized by convolving a gaussian white-noise image with a band-pass filter. The peak spatial frequency of the band-pass filter decreases over time, producing a gradual change in scale of texture elements. The *x*–*t* slice illustrates the lack of consistent displacement information. Because each frame is generated from independent white noise, the resulting optic-flow field is random, and thus cannot be used to estimate expansion rate. **b**, Limited-lifetime random dots stimulus. Dots appear asynchronously in random locations, move according to the global expansion pattern for five frames, and then disappear. For this stimulus, the optic-flow field may be used to estimate expansion rate, but there is minimal scale-change information. **c**, Deterministic texture stimulus. Each frame is a re-scaled replica of a single image of band-pass-filtered gaussian white noise. Both optic flow and scale changes provide information about the expansion rate.