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Isotope exchange reactions with radiolabeled sulfur compounds in anoxic seawater

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Abstract. The isotope exchange between ^{35}S-labeled sulfur compounds of sulfate (SO_4^{2-}), elemental sulfur (S^0), polysulfide (S_n^{2-}), hydrogen sulfide (ΣHS^-: $H_2S + HS^- + S^{2-}$), iron sulfide (FeS), and pyrite (FeS_2) was studied at pH 7.6 and 20 °C in anoxic, sterile seawater. Isotope exchange was observed between S^0, S_n^{2-}, ΣHS^-, and FeS, but not between ^{35}S labeled SO_4^{2-} or FeS_2 and the other sulfur compounds. Polysulfide mediated the isotope exchange between S^0 and bisulfide (HS^-). The isotope exchange between S^0 and S_n^{2-} reached 50% of equilibrium within < 2 min while exchange between S_n^{2-} and HS^- approached equilibrium within 0.5–1 h. In all the experiments ΣHS^- revealed a fraction exchange from 0.79 to 1.00. Isotope exchange between S^0 and FeS took place only via S_n^{2-} and/or HS^-. The isotope exchange between iron sulfide and the other sulfur compounds was not complete within 24 h as shown by a fraction exchange of 0.07–0.83. This lack of equilibrium (fraction exchange < 1) was due to the isotope exchange between dissolved compounds and surfaces of sulfur particles. The isotopic exchange reactions limit the usefulness of radiotracers in process studies of the inorganic sulfur species. Exchange reactions will also affect the stable isotope distribution among the sulfur species. The kinetics of the isotopic exchange reactions, however, depend on both pH and temperature.

Introduction

Stranks & Wilkins (1957) defined an exchange reaction as *a chemical reaction in which the atoms of a given element interchange between two or more chemical forms of this element*. The term, isotope exchange reaction, is used here since such an exchange reaction can only be detected if the chemical forms are labeled with an isotope.

Voge & Libby (1937) and Voge (1939) were the first to study sulfur exchange reactions by use of radiolabeled compounds. They observed isotope exchange at 100 °C between S^0, S_n^{2-}, and ΣHS^-, but no exchange into SO_4^{2-}, and McKay (1938) described this kind of isotope exchange in mathematical terms.

In studies of sulfur geochemistry, exchange reactions between SO_4^{2-} and

ΣHS^- have been demonstrated only at temperatures above 200 °C (Robinson 1973; Sakai & Dickson 1978; Ohmoto & Lasaga 1982; Uyama et al. 1985). In the temperature range from 0 to 25 °C, the expected equilibrium constants for several exchange reactions involving the sulfur isotopes ^{32}S and ^{34}S have been calculated by means of statistical mechanics (Tudge & Thode 1950). These calculations showed that exchange reactions between sulfur compounds should theoretically result in a 1% increase of the heavy isotope in the more oxidized compound with the exception of the exchange between SO_4^{2-} and H_2S. In this reaction SO_4^{2-} should be enriched in the heavy isotope by about 8.5%.

Sulfur isotope exchange reactions may also be important for the biogeochemical sulfur cycle in the low-temperature range. Jørgensen and coworkers (1979) studied sulfur transformations in the chemocline of a stratified salt lake. They observed a rapid formation of radioactive S_n^{2-} and ΣHS^- from radiolabeled elemental sulfur ($^{35}S^0$) and argued that isotope exchange was the most important mechanism for this formation. Fry and coworkers (1984; 1988) observed an inverse isotopic fractionation during microbial oxidation of ΣHS^- to S^0, i.e. enrichment of S^0 with the heavy isotope. They argued that this effect was caused by an isotope equilibrium reaction. We have recently found strong evidence for isotope exchange from studies of transformations of ^{35}S-labeled sulfur compounds in marine sediment slurries and undisturbed sediment cores (Fossing & Jørgensen in prep.)

We have therefore done experiments at 20 °C in anoxic seawater at its natural pH of 7.6 to study isotope exchange between radiolabeled sulfur compounds. The sulfur radiotracers were all prepared in the laboratory and exchange was studied between: SO_4^{2-}, S^0, S_n^{2-}, ΣHS^-, FeS, and FeS_2.

Material and methods

The ^{35}S isotope exchange was studied in time course experiments of up to 24 h. Experiments were done at pH 7.6 and 20 °C in anoxic, sterile seawater (15–20‰) and $^{35}S^0$, $\Sigma H^{35}S^-$, and $Fe^{35}S_2$ were used as radiotracers.

Preparation of radiolabeled sulfur compounds

Radiolabeled S^0 was obtained commercially (Amersham Corp.) while $Fe^{35}S_2$ and $\Sigma H^{35}S^-$ were synthesized in the laboratory from $^{35}S^0$.

$^{35}S^0$

Elemental sulfur was dissolved in toluene when it was shipped. The toluene was evaporated and the $^{35}S^0$ was redissolved in acetone (anal. grade, Merck).

Just before injection, $^{35}S^0$ in acetone was mixed with anoxic seawater, 1:10. A colloidal suspension of 1 μm size S^0 granules formed immediately. Approximately 150 kBq (4 μCi) of $^{35}S^0$ in 5 μl suspension was used for each $^{35}S^0$ labeled experiment.

$\Sigma H^{35}S^-$

Radioactive ΣHS^- was prepared by chromium reduction of $^{35}S^0$ and was trapped in 0.01 N NaOH as $^{35}S^{2-}$ (Fossing & Jørgensen 1989a). After reduction, the concentration of S^{2-} in the trap was about 25 mM. The sulfide solution was kept anoxic to limit the oxidation of $^{35}S^{2-}$. Only 2% of the $^{35}S^{2-}$ was oxidized during this storage of which 1.9% was oxidized to $^{35}S^0$ and 0.1% to $^{35}SO_4^{2-}$. About 150 kBq of $^{35}S^{2-}$ in 20 μl NaOH was added to each $H^{35}S^-$ labeled experiment. The added radiotracer did not alter the concentration of ΣHS^- or the pH. The radioactive S^{2-} became protonated to approx. 80% HS^- and 20% H_2S immediately after injection.

$Fe^{35}S_2$

Radioactive pyrite was prepared from $^{35}S^{2-}$ with S^{2-} carrier, ferrous iron, and elemental sulfur (Berner 1969, 1970; Sweeney & Kaplan 1973; Fossing & Jørgensen 1989a). The $^{35}S^{2-}$, prepared by chromium reduction of $^{35}S^0$, was mixed with 6 mmol Fe^{2+} and an equal amount of carrier sulfide in 50 ml of boiling distilled water whereby FeS precipitated. Flowers of sulfur was added to the precipitate and pyrite formed after four days of ageing at 65 °C plus four days at 85 °C. Excess FeS and ΣHS^- were removed by boiling with HCl while S^0 was removed by CS_2 extraction. After cleaning, X-ray crystallography of the dried sulfur mineral showed that only FeS_2 was detectable. More than 99.9% of the radioactivity was $Fe^{35}S_2$. Ten mg of the radioactive pyrite (40 kBq mg^{-1}) was sonicated with 2 ml of anoxic distilled water. About 0.5 ml of this suspension was added when a $Fe^{35}S_2$ labeled experiment was started.

Experimental

Non-labeled sulfur compounds were prepared as described by Fossing & Jørgensen (1989b). Elemental sulfur was used as a colloidal suspension in 1:10, acetone:water. A 0.5 M stock solution of ΣHS^- was prepared from $Na_2S \cdot 9H_2O$ dissolved in anoxic distilled water at pH 7.6 adjusted by 0.1 N HCl. Polysulfide formed during the experiment by reaction between S^0 and HS^- (Schwarzenbach & Fischer 1960; Chen & Morris 1972; Millero 1986). Iron sulfide was precipitated by mixing ΣHS^- with Fe^{2+} solutions and FeS_2 was synthesized from this precipitate by reaction with S^0.

Isotope exchange experiments were all done in anoxic, filter sterilized seawater; 17.7 ± 0.5 mM SO_4^{2-} (n = 10). A glass container (see below) was used as the reaction vessel for the following experiments:

I. Radiolabeled SO_4^{2-} formation
The following exchange reaction was tested:

$$^{35}S^0 + SO_4^{2-} \rightleftharpoons S^0 + {}^{35}SO_4^{2-} \qquad (1)$$

The oxidation of $^{35}S^0$ to $^{35}SO_4^{2-}$ and the possible exchange between S^0 and SO_4^{2-} was followed in a time course experiment for 144 h. The experiment was started by adding 0.1 μmol $^{35}S^0$ ml^{-1}. Subsamples were analyzed for concentrations and radioactivities of $^{35}S^0$ and $^{35}SO_4^{2-}$ as well as for total radioactivities.

II. Polysulfide formation
The following exchange reaction was tested:

$$\underbracket{\mathbf{S{-}S{-}S{-}S{-}S{-}S{-}S{-}S}} + 2HS^- \rightleftharpoons 2\,{}^-S{-}\mathbf{S{-}S{-}S{-}S}^- + 2H^+ \qquad (2)$$

where **S** (bold type) is ^{35}S, $\underbracket{S{-}S{-}S{-}S{-}S{-}S{-}S{-}S}$ symbolizes the ring structure of elemental sulfur (S^0 or S_8) and $^-S{-}\mathbf{S{-}S{-}S{-}S}^-$ is the radiolabeled polysulfide ($^{35}S_n^{2-}$). The S_n^{2-} is actually a complex mixture of polysulfide with S-chain length from n = 2–5 but with S_4^{2-} and S_5^{2-} as the dominating species (Schwarzenbach & Fischer 1960; Giggenbach 1972). The formation of $^{35}S_n^{2-}$ was studied during a 1 h time course experiment by adding 0.04 μmol $^{35}S^0$ ml^{-1} to seawater with 2.4 mM ΣHS^-. The great excess of ΣHS^- forced the equilibrium reaction (2) to the right. The analyzed sulfur compounds comprised: S^0, S_n^{2-}, ΣHS^- and SO_4^{2-}.

III. Isotope exchange between S^0, S_n^{2-}, and HS^-
The following exchange reactions were tested:

$$\underbracket{\mathbf{S{-}S{-}S{-}S{-}S{-}S{-}S{-}S}} + 2HS^- \rightleftharpoons 2\,{}^-S{-}\mathbf{S{-}S{-}S{-}S}^- + 2H^+ \rightleftharpoons$$
$$\underbracket{S{-}\mathbf{S{-}S{-}S{-}S{-}S{-}S{-}S}} + 2HS^- \qquad (3)$$

Final concentrations of 1 μmol S^0 ml^{-1} and 1 mM ΣHS^- were preincubated for 1 h during which S_n^{2-} formed. The experiment was started by adding trace amounts of $^{35}S^0$. After isotopic equilibrium was reached, we increased the concentration of ΣHS^- 3-fold. The time course of the redistribution of ^{35}S was followed for 1.5 h.

IV. Isotope exchange between HS^- and FeS

The following exchange reactions were tested:

$$H^{35}S^- + FeS + H^+ \rightleftharpoons H^{35}S^- + Fe^{2+} + HS^- \rightleftharpoons HS^- + Fe^{35}S + H^+ \tag{4a}$$

Three experiments were done with FeS that had been aged in sealed glass bottles for 3 days, 5 months, and 2.5 years. Final concentrations of 10 μmol FeS ml^{-1} and 1.3 mM ΣHS^- were mixed. The $H^{35}S^-$ tracer was added after a 24 h preincubation. Subsamples were taken in parallels of 5 after 0, 1.5, and 24 h, respectively, and the average radioactivities and concentrations of ΣHS^-, FeS, and S^0 + FeS_2 were calculated.

VA and VB. Isotope exchange between S^0, S_n^{2-}, HS^-, and FeS

In experiment VA the following overall exchange reactions were tested:

$$2{}^{35}S^0 + FeS + HS^- \rightleftharpoons 2S^0 + Fe^{35}S + H^{35}S^- \tag{5}$$

Final concentrations of 0.01 μmol ΣHS^- ml^{-1}, 6.0 μmol FeS ml^{-1} freshly prepared, and 0.4 μmol S^0 ml^{-1} were mixed. Radioactive S^0 was added after preincubation for 1 h. We increased the ΣHS^- concentration about 9 fold, 1.3 h after the experiment was started. After isotopic equilibrium was established the concentration of FeS was increased to 17.7 μmol ml^{-1}. After isotopic equilibrium had been reached once again, ΣHS^- was added to a final concentration of 1.3 μmol ml^{-1}. The experiment was terminated after 9.5 h.

In experiment VB, contamination with ΣHS^- was carefully avoided and the following overall exchange reaction was tested:

$$^{35}S^0 + FeS \rightleftharpoons S^0 + Fe^{35}S \tag{6a}$$

The precipitated FeS was washed thoroughly with boiling distilled water to remove traces of ΣHS^-. Only 0.8 μmol FeS ml^{-1} was used, mixed with 1 μmol S^0 ml^{-1}. One hour after the experiment was started, ΣHS^- was added to this mixture to a final concentration of 0.9 μmol ΣHS^- ml^{-1}. This addition of ΣHS^- was expected to produce S_n^{2-}. The experiment was terminated after 4 h.

VI. Isotope exchange between HS^- and FeS_2

The following exchange reaction was studied:

$$H^{35}S^- + FeS_2 \rightleftharpoons HS^- + Fe^{35}SS \tag{7a}$$

The isotope exchange between HS^- and FeS_2 was investigated in two experiments. One experiment was labeled with $\Sigma H^{35}S^-$ and the other with $Fe^{35}S_2$ after a preincubation of 24 h. The concentrations of ΣHS^- and FeS_2 in the two experiments were 4.44 and 4.71 mM ΣHS^{-1}, respectively, and 17.18, and 14.38 μmol S_{eqv} of FeS_2 ml^{-1}. Subsamples, in parallels of 5, were analyzed in each experiment after 0, 1.5, and 24 h and the average concentrations and radioactivities were calculated.

VII. Pyrite formation

The formation of FeS_2 from S^0 and FeS was examined at 20 °C during incubation for up to 24 h:

$$S^0 + FeS \longrightarrow FeS_2 \tag{8}$$

Radiolabeled S^0 was added to a suspension of 5.4 μmol FeS ml^{-1} to equivalent concentrations. After 0, 1.5, and 24 h, subsamples in parallels of 5 were taken and the average radioactivities and concentrations of S^0, S_n^{2-}, ΣHS^-, FeS, and FeS_2 were determined.

The reaction vessel

A glass cylinder was filled with 0.5 liter filter sterilized (0.22 μm) seawater. The water was bubbled with N_2 via an O_2-scrubber for 30 min before the reaction vessel was tightly closed (without headspace) with a movable Teflon piston fitted inside the cylinder. This piston was equipped with a stopcock through which samples were taken or solutions were added. The piston followed any changes in volume. The glass cylinder was placed on a magnetic stirrer and the content was stirred continuously by a small magnet. A 0.1 NHCl solution was used to adjust the sea water to pH 7.6. The appropriate sulfur compounds were added and the solution was preincubated for 1–24 h. The experiments were started by injecting radiotracer ($^{35}S^0$, $\Sigma H^{35}S^-$, or $Fe^{35}S_2$) through the stopcock.

The subsampling began immediately after the radiotracer was added. Subsampling was frequent during the first 10–15 min of an experiment. The sampled sulfur compounds were immediately separated and preserved for later analysis.

Separation of sulfur compounds

Figure 1 shows the setup for a rapid separation of the following sulfur compounds: SO_4^{2-}, S^0, S_n^{2-}, ΣHS^-, FeS, and FeS_2. By this setup, all of the sulfur compounds were separated within less than 4 min. Sulfate was only measured a few times during an experiment to check that no $^{35}SO_4^{2-}$ was formed.

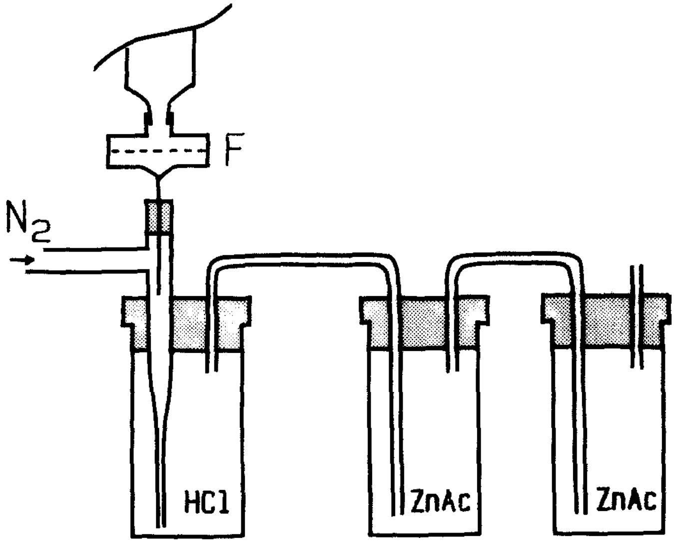

Fig. 1. Setup for a rapid separation of SO_4^{2-}, S^0, S_n^{2-}, ΣHS^-, FeS, and FeS_2. The filter unit, *F*, is connected to the first vial by the use of a needle that penetrates a self-sealing rubber stopper . The first vial contains 5 ml of 3 N HCl when the setup is used for ΣHS^- distillation and is empty for FeS distillation. The second and third vial each contains 10 ml of 2% ZnAc with a drop of antifoam. A flow of N_2 carries the evolved H_2S to the two ZnAc vials.

Five ml (volume determined by weight and density) was sampled from the container through the stopcock by use of a syringe. The solution was filtered through a 0.22 μm filter directly into the first scintillation vial (vol. 20 ml) by use of a needle that penetrated a selfsealing rubber stopper. The time of subsampling was recorded when half the sample had passed the filter. More than 99.6 ± 0.1% ($n = 3$) of the solid sulfur compounds, S^0, FeS, and FeS_2, were retained by the filter. Only the dissolved sulfur species, S_n^{2-} and ΣHS^-, entered the first vial that contained 5.0 ml of 3 N HCl. Here an acid distillation took place:

$$HS^- + H^+ \longrightarrow H_2S \tag{9}$$

$$S_n^{2-} + 2H^+ \longrightarrow H_2S + (n - 1)S^0 \tag{10}$$

A flow of N_2 carried the formed H_2S gas to a second vial where it was trapped as ZnS in 10 ml of 2% zinc acetate (ZnAc) with a drop of antifoam. A third vial with 10 ml of 2% ZnAc ensured that all H_2S was trapped. This vial never contained any sulfide after the distillations. Distillation continued for 30 min with a recovery of 94.3 ± 3.5% ($n = 3$) of the ΣHS^- that entered the first vial.

After this filtration and distillation,
— S^0, FeS, and FeS_2 were recovered on the filter,
— $(n - 1)/n$ S^0 from S_n^{2-} was recovered in the first vial,
— $\Sigma HS^- + 1/n$ S^{2-} from S_n^{2-} was recovered as ZnS in the second vial.

The filter with the solid sulfur compounds was washed with 10 ml of anoxic distilled water to ensure a complete removal of the dissolved pools. The filter unit was then connected to a new set of scintillation vials with the first vial empty and the next two with ZnAc. Ten ml of anoxic 3 N HCl was flushed through the filter, FeS was thereby dissolved, and H_2S formed. The N_2 flow carried H_2S to the second vial where the H_2S was trapped as ZnS.

Remaining on the filter was S^0 and FeS_2. The filter was washed with 10 ml of anoxic distilled water and S^0 was dissolved by flushing 5 ml CS_2 through the filter. Controls with $^{35}S^0$ suspension showed that only 0.4 ± 0.2% ($n = 6$) of the S^0 remained on the filter after extraction with CS_2. Remaining pyrite was reduced with Cr^{2+} and H_2S was trapped in 10 ml of 5% ZnAc (Zhabina & Volkov 1978; Canfield et al. 1986; Fossing & Jørgensen 1989b). Controls showed no detectable isotope exchange between $^{35}S^0$ and FeS or FeS_2 during separations. There is no isotope exchange between elemental sulfur and CS_2 (Cooley et al. 1939).

Elemental sulfur originating from the polysulfide was filtered through a 0.22 μm filter, as HCl otherwise quenched the radioactive sample. The filter was dried and placed again in the first vial for $^{35}S^0$ counting together with some S^0 which adhered to the plastic wall. The formation of $^{35}SO_4^{2-}$ was checked on samples as $BaSO_4$ after precipitation with $BaCl_2$.

Determination of concentrations and radioactivities

Sulfate concentration was determined gravimetrically after precipitation with Ba^{2+} (American Public Health Association 1971). The concentrations of elemental sulfur and polysulfide sulfur was determined spectrophotometrically after cyanolysis as described by Troelsen & Jørgensen (1982). The concentration of ΣHS^-, FeS, and FeS_2 (measured in sulfur equivalents: S_{eqv}) were determined spectrophotometrically (Cline 1969) on subsamples of ZnS from the ZnAc traps.

All radioactivities were determined in a liquid scintillation analyzer (Tri-Carb 2200 CA, Packard). Radiolabeled sulfate was measured from the precipitated $BaSO_4$ after it was washed once, mixed with 5 ml of distilled water plus 5 ml of scintillation fluid (Dynagel, Baker Chemicals) and gelled. The CS_2 was evaporated from the extracted elemental sulfur and S^0 was redissolved in 5 ml scintillation fluid. Polysulfide sulfur on filters was also redissolved in scintillation fluid. Before the radioactivity of sulfur was counted, 5 ml of distilled water was added and the mixture was gelled. Radioactivities of ΣHS^-, FeS, and FeS_2 were determined from 10 ml of ZnS precipitate which was mixed with 10 ml of scintillation fluid and gelled.

Specific radioactivity (S_a) of sulfur compound *a* was at any time *t* calculated from absolute radioactivity (A^*) and concentration (A)

$$S_a = \frac{A^*}{A} \tag{i}$$

The specific radioactivities of the sulfur compounds were compared and this way it was possible to describe the ^{35}S distribution at any time *t* relative to isotopic equilibrium. At isotopic equilibrium the isotope must be distributed in proportion to concentrations and the specific radioactivities were consequently expected to equal (Myers & Prestwood 1951; Johnston 1977)

$$\frac{A^\infty}{A} = \frac{B^\infty}{B} = \frac{C^\infty}{C} \tag{ii}$$

where A^∞, B^∞, and C^∞ are the absolute radioactivity at time $t = \infty$ (i.e. equilibrium) of compound *a*, *b*, and *c*, respectively. Concentrations did not change during the exchange experiments and the specific radioactivites therefore revealed if ^{35}S-atoms were brought into equilibrium by isotope exchange.

Results and discussion

Radiolabeled SO_4^{2-} was not observed in any of the experiments within 12 h of incubation. In experiment I, where $^{35}S^0$ was added to seawater, we observed a linear decrease of about 30% in both $^{35}S^0$ and total radioactivity within 3 h (data not shown). No other radiolabeled sulfur compounds were observed during this period. The decrease in $^{35}S^0$ was due to gradual adhesion of S^0 granules to the glass wall of the container. This artifact has been corrected for in the experimental data. Radioactive SO_4^{2-} was first detectable after 12–24 h and increased to 100% of total activity after 144 h. No change in SO_4^{2-} concentration was observed. The increase in $^{35}SO_4^{2-}$ was caused by a slow oxygenation of $^{35}S^0$, presumably from trace amounts of O_2 that leaked into the container. A similar experiment, but using $^{35}SO_4^{2-}$ instead of $^{35}S^0$, showed that ^{35}S remained in the sulfate pool throughout the 144 h (unpubl. results). We therefore conclude that isotope exchange does not take place between S^0 and SO_4^{2-} at room temperature. As the other time course experiments ran only for a few hours, O_2 penetration into the container was too small to influence the data obtained.

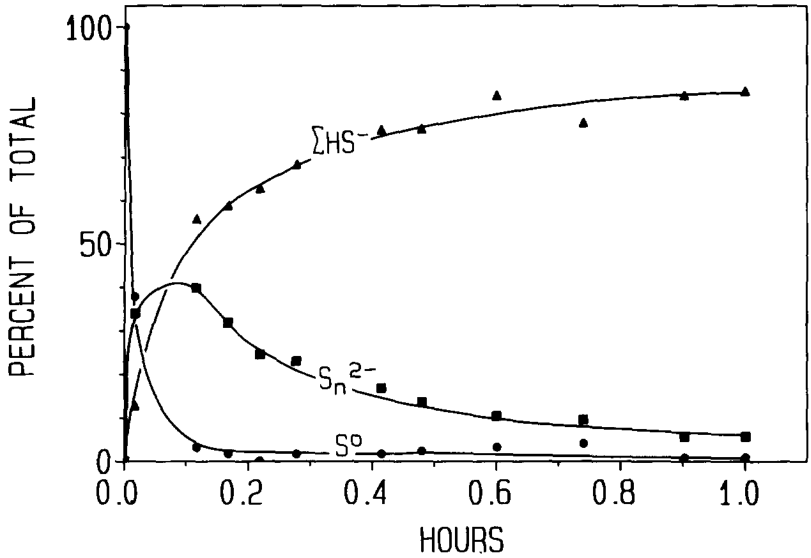

Fig. 2. Time course of S_n^{2-} formation from $^{35}S^0$ and ΣHS^-, experiment II. The radioactivity is expressed in percent of total activity which equals the initial $^{35}S^0$.

Voge & Libby (1937) and Voge (1939) showed that no isotope exchange proceeded between SO_4^{2-} and HS^- at 100 °C during a 36 h period. Others have shown that only at > 200 °C does significant exchange between SO_4^{2-} and ΣHS^- take place (Robinson 1973; Sakai & Dickson 1978; Ohmoto & Lasaga 1982; Uyama et al. 1985). Consequently, we did not expect SO_4^{2-} to participate in any isotope exchange at room temperature and only checked for $^{35}SO_4^{2-}$ a few times during subsequent experiments to verify that it was not formed.

Isotope exchange between S^0, S_n^{2-}, and HS^-

Experiment II revealed a rapid polysulfide formation when trace amounts of $^{35}S^0$ were added to a ΣHS^- solution (Fig. 2):

$$(n-1)S^0 + HS^- \longrightarrow S_n^{2-} + H^+ \qquad (11)$$

The polysulfide formation was accompanied by an isotope exchange. This resulted in an isotopic equilibration between S^0, S_n^{2-}, and HS^- within 1 h according to equation (3):

$$\underbrace{\text{S–S–S–S–S–S–S–S}} + 2HS^- \rightleftharpoons 2\,{}^-\text{S–S–S–S–S}^- + 2H^+$$
$$\rightleftharpoons \underbrace{\text{S–S–S–S–S–S–S–S}} + 2HS^- \qquad (3)$$

The concentrations of both S^0 and S_n^{2-} were below detection limit, 5 nmol ml^{-1}. Hence, the rapid formation of S_n^{2-} was revealed only by the increase in S_n^{2-} radioactivity. The initial increase of $^{35}S_n^{2-}$ was followed by an exponential decrease when the isotope exchanged into ΣHS^- which was much larger than the other two sulfur pools (Fig. 2). The fraction of the total radioactivity which was exchanged from S^0 and S_n^{2-} into ΣHS^- within 1 h was 0.85 or 85%. This fraction exchange, F, is defined as (Myers & Prestwood 1951):

$$F(a) = \frac{A - A^0}{A^\infty - A^0} = \frac{S_a - S_a^0}{S_a^\infty - S_a^0} \qquad \text{(iii)}$$

where $F(a)$ is the fraction exchange of compound a, A is the absolute radioactivity, and S_a is the specific radioactivity at time $= t$. The superscripts 0 and ∞ refer to $t = 0$ and $t = \infty$, respectively. Consequently, when complete isotopic equilibrium is reached between compound a, b, and c then $F(a)$, $F(b)$, and $F(c)$, all equal 1.00. Isotopic steady state distributions with F-values < 1 are referred to as pseudo-equilibria. Conditions leading to pseudo-equilibrium are discussed below.

In experiment III we also observed isotope exchange between S^0 and HS^-. Isotopic pseudo-equilibrium was reached within 0.5 h (Fig. 3A). We measured constant concentrations of S^0, S_n^{2-}, and ΣHS^- of $0.43 \pm 0.07\,\mu$mol ml^{-1}, $0.53 \pm 0.07\,\mu$mol S_{eqv} ml^{-1}, and 0.99 ± 0.08 mM ($n = 10$), respectively, throughout the first hour of the experiment. Consequently, based on equation (ii) we expected roughly a 1:1:2 distribution of the isotope between S^0, S_n^{2-}, and ΣHS^-, which was also observed (Fig. 3A). The fraction exchange for S^0, S_n^{2-}, and HS^- were 0.92 ± 0.03, 0.99 ± 0.01, and 0.87 ± 0.02 ($n = 3$), respectively, as calculated from the asympthotic values just before 1 h (Fig. 3B). The isotope exchange to HS^- was therefore not complete based on concentrations. The ionactivity of HS^- was not calculated in these experiments. As the activity coefficient is significantly less than 1 (e.g. Davison & Heaney 1980), a more accurate calculation of equilibrium can be obtained based on ionactivity calculations.

The concentration of ΣHS^- was increased to 2.80 ± 0.10 mM ($n = 7$) after 1 h and immediately the radioisotope distribution shifted (Fig. 3A). The increase in ΣHS^- radioactivity was inversely related to the exponential decrease in both S^0 and S_n^{2-} radioactivity. Isotopic pseudo-equilibrium was reached after <0.5 h. The distribution of the isotope was expected to be 2:2:13 based on the new concentrations of S^0, S_n^{2-}, and ΣHS^- but only 60% of the total radioactivity was found in ΣHS^-. We calculate, based on this observation, a fraction exchange for HS^- of 0.79 (0.60/0.76).

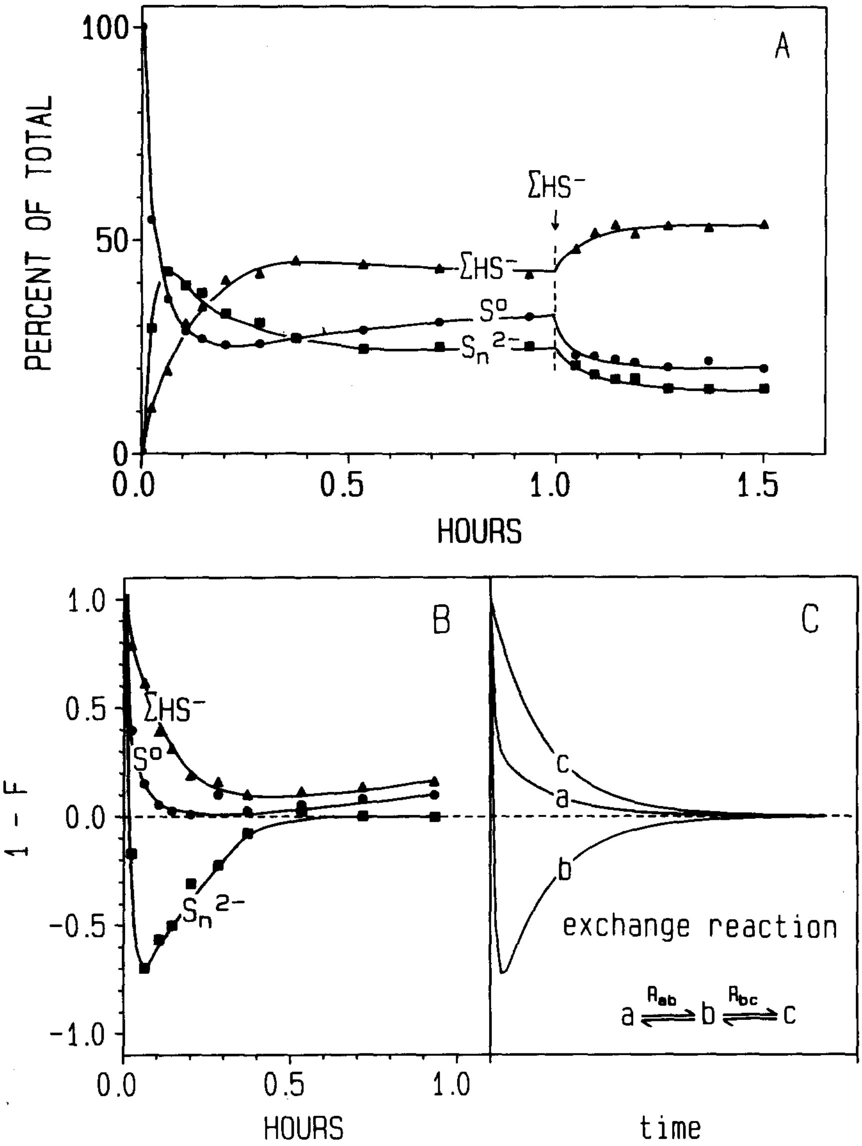

Fig. 3. Time course of isotope exchange between S^0, S_n^{2-}, and HS^-. A: Experiment III, $^{35}S^0$ tracer. The radioactivity is expressed in percent of total activity which equals the initial $^{35}S^0$. The chemical concentrations of S^0, S_n^{2-}, and ΣHS^- were constant during 0–1 h and again during 1–1.5 h. The arrow shows time when the ΣHS^- concentration was stepped up. B: The first hour of experiment III. At equilibrium, $(1 - F)$ equals zero according to the definition (see text). C: Theoretical $(1 - F)$ plot for isotope exchange between three compounds, a, b, and c, with concentration of A, B, and C, provided that the rate $R_{ab} \gg R_{bc}$, $R_{ac} = 0$, and $2A = 2B = C$.

Complete isotope exchange between S^0 and HS^- was observed within 1 h by Voge (1939). His experiments were all done at 90–100 °C and S_n^{2-} was formed by the addition of S^0 to a ΣHS^- solution. Isotope exchange was studied at 0 and 25 °C at a pressure of <20 atm in experiments where radiolabeled S^0 was dissolved in liquid H_2S (Mickelsen et al. 1966). In this way it was possible to prevent S_n^{2-} from being formed and no radioisotope exchange was observed with the solvent (H_2S) in these experiments. On the other hand, the isotope exchange between S^0 and HS^- was complete within 10–90 min as soon as traces of $((C_2H_5)_3NH)SH$ were added to the experimental solution. The triethylammonium hydrosulfide is highly effective in opening S_8 rings. This ring-opening caused the formation of S_n^{2-} which was responsible for the observed exchange reaction between S^0 and HS^- (Mickelsen et al. 1966).

It is therefore likely that the presence of S_n^{2-} is essential for any exchange between S^0 and HS^-. We used a mathematical model (Abell et al. 1957; Fleck 1972) to calculate the kinetics of this isotopic exchange and to demonstrate the role of S_n^{2-}. The model is based on concentrations rather than on ionactivities.

The rate constants for the exchange between three compounds a, b, and c are given by R_{ab}, R_{ac}, and R_{bc}. The concentrations and radioactivities of a, b, and c are given by A, B, and C and A^*, B^* and C^*, respectively. The concentrations are constant and so is the total radioactivity. The specific activities for compound a, b, and c are S_a, S_b, and S_c, respectively (see equation (i)).

The change in radioactivities of compound a, b and c is then expressed as:

$$\mathrm{d}A^*/\mathrm{d}t = R_{ab}(S_b - S_a) + R_{ac}(S_c - S_a) \tag{iv-a}$$

$$\mathrm{d}B^*/\mathrm{d}t = R_{ab}(S_a - S_b) + R_{bc}(S_c - S_b) \tag{iv-b}$$

$$\mathrm{d}C^*/\mathrm{d}t = R_{ac}(S_a - S_c) + R_{bc}(S_b - S_c) \tag{iv-c}$$

A solution to the differential equation, $\mathrm{d}A^*/\mathrm{d}t$, is expressed by

$$1 - F(a) = Q_{a1}\exp(-m_1 t) + Q_{a2}\exp(-m_2 t) \tag{v}$$

where $F(a)$ is the fraction exchange of compound a at time t, Q_{a1}, Q_{a2}, m_1, and m_2 are constants and $Q_{a1} + Q_{a2} = 1$. Similar solutions to the other two differential equations, $1 - F(b)$ and $1 - F(c)$, are obtained when a in equation (v) is substituted with b and c, respectively.

A plot of $\log(1 - F)$ vs. t normally yields a curve which is composed of two straight lines. The slopes of these lines are given by m_1 and m_2 and they intercept the *y*-axis at Q_1 and Q_2, respectively. The values of Q_1, Q_2, m_1, and m_2 can also be calculated from the rate constants, R_{ab}, R_{ac}, and R_{bc}, whereas the rate constants *cannot* be calculated from Q_1, Q_2, m_1, and m_2 (cf. Abell et al. 1957). Consequently, we were not able to calculate the exchange rates between S^0, S_n^{2-}, and HS^- from the observations done in experiment III.

Instead we plotted $(1 - F)$ vs. t for the three compounds, S^0, S_n^{2-}, and ΣHS^-, for the first hour of Experiment III (Fig. 3B). The S_n^{2-} plot starts at 1.0 (implicit from definition), becomes negative, and then asymptotically approaches zero from the negative side. The S^0 and ΣHS^- plots both start at 1.0 and asymptotically approach zero from the positive side. Figure 3C shows a computer simulation of the exchange curves obtained by observing $F(a)$, $F(b)$, and $F(c)$ (cf. Abell et al. 1957) when a is the initially labeled compound, $R_{ab} \gg R_{bc}$, $R_{ac} = 0$, and $2A = 2B = C$; i.e.

$$a \underset{}{\overset{R_{ab}}{\rightleftharpoons}} b \overset{R_{bc}}{\rightleftharpoons} c \tag{12}$$

In experiment III, a, b, and c represent S^0, S_n^{2-}, and ΣHS^-, respectively. As $R_{ab} \gg R_{bc}$ the isotope builds up in compound b faster than it exchanges with compound c. Consequently, $1 - F(b)$ becomes negative. The similarity between Fig. 3B and 3C shows that the isotope exchange between S^0 and ΣHS^- mainly proceeds through S_n^{2-} and that the exchange rate between S^0 and S_n^{2-} is higher than the exchange rate between S_n^{2-} and HS^-. This is to be expected because S^0 is neutral whereas S_n^{2-} and HS^- are negative and consequently should repel each other. Hence, one of the exponential terms in the $(1 - F(S^0))$ plot (Fig. 3B) dominates during the first minutes of the experiment and we can therefore estimate the half-time ($t_{1/2}$) of the isotope exchange between S^0 and S_n^{2-} (Myers & Prestwood 1951; Fleck 1972). We calculated that 1.8 min was the time necessary for the fraction exchange to reach 0.5 or 50% equilibration.

Polysulfides with various n also exchange (Giggenbach 1972) and so do S-atoms within the S_n^{2-}-chains by self exchange (Voge 1939; Körös et al. 1957). The equilibria between H_2S, HS^-, and S^{2-} imply that ^{35}S-atoms also are exchanged between these sulfide species during the experiments. However, isotope exchanges of this kind could not be observed the way our experiments were designed.

Isotope exchange with FeS

Experiment IV was done to investigate isotope exchange between ΣHS^- and FeS particles of various age. No S^0 or FeS_2 were detectable and no significant

Table 1. Isotope exchange between HS^- and FeS particles of various age.

Age of FeS	FeS concentration in % of AVS	Fraction exchange of FeS	
		1.5 hours	24 hours
3 days	88.1 ± 3.5	0.073 ± 0.004	0.230 ± 0.033
5 months	88.5 ± 7.5	–	0.159 ± 0.059
2.5 years	89.2 ± 4.0	0.071 ± 0.010	0.233 ± 0.026

The concentration of FeS in percent of total AVS (FeS + ΣHS^-) is presented. The fraction exchange of FeS is calculated after 1.5 and 24 h, respectively. Numbers are the mean of 5 and 10 measurements ± s.d. of the mean, for fraction exchange and concentration, respectively.

changes in concentrations of ΣHS^- and FeS were observed during a 24 h period ($p < 0.01$; Student's t-test; $f = 8$). The experiment showed a fraction exchange of up to 0.073 ± 0.004 ($n = 5$) for FeS during 1.5 h (Table 1). This fraction exchange increased up to 0.233 ± 0.026 ($n = 5$) after 24 h of incubation. We did not observe any correlation between the age of FeS and the kinetics of isotope exchange.

Since F(FeS) was < 1, the isotope exchange did not run to completion within 24 h. This could be explained from the exchange mechanism between solid particles (FeS) and dissolved compounds (ΣHS^-). The sulfur isotopes were not distributed in the same phase when ΣHS^- and FeS were mixed. Consequently, an isotopic pseudo-equilibrium was reached between S-atoms on the surfaces of the solid particles and the aqueous compounds, which were labeled with ^{35}S. A pseudo-equilibrium can result from a relatively rapid saturation of the surface of the solid particles and a slow self diffusion of this isotope into the particles (Myers & Prestwood 1951; Johnston 1977). The exchanging isotopes are therefore not distributed between the compounds in the same proportions as the concentrations of these compounds predict. Instead, the isotope distribution depends on the surface to volume ratio of the solid particles. Corrections for ionactivities in this experiment are not significant due to the large excess of HS^-.

It is puzzling that no age dependent isotope effect was observed, as the 2.5 years old FeS crystals are expectedly larger and more stable that the freshly prepared FeS. This may be because the FeS remains in an amorphous form with a very large surface to volume ratio and thus a high capacity for isotope exchange.

In Experiment VA and VB, studies of isotope exchange were done between the two solid sulfur compounds, S^0 and FeS. Experiment VB, in which ΣHS^- was undetectable, did not show any isotope transfer from $^{35}S^0$ to FeS during 0–1 h (Fig. 4B), i.e. exchange reaction (6a) *did not* proceed

$$^{35}S^0 + FeS \not\rightleftarrows S^0 + Fe^{35}S \qquad (6b)$$

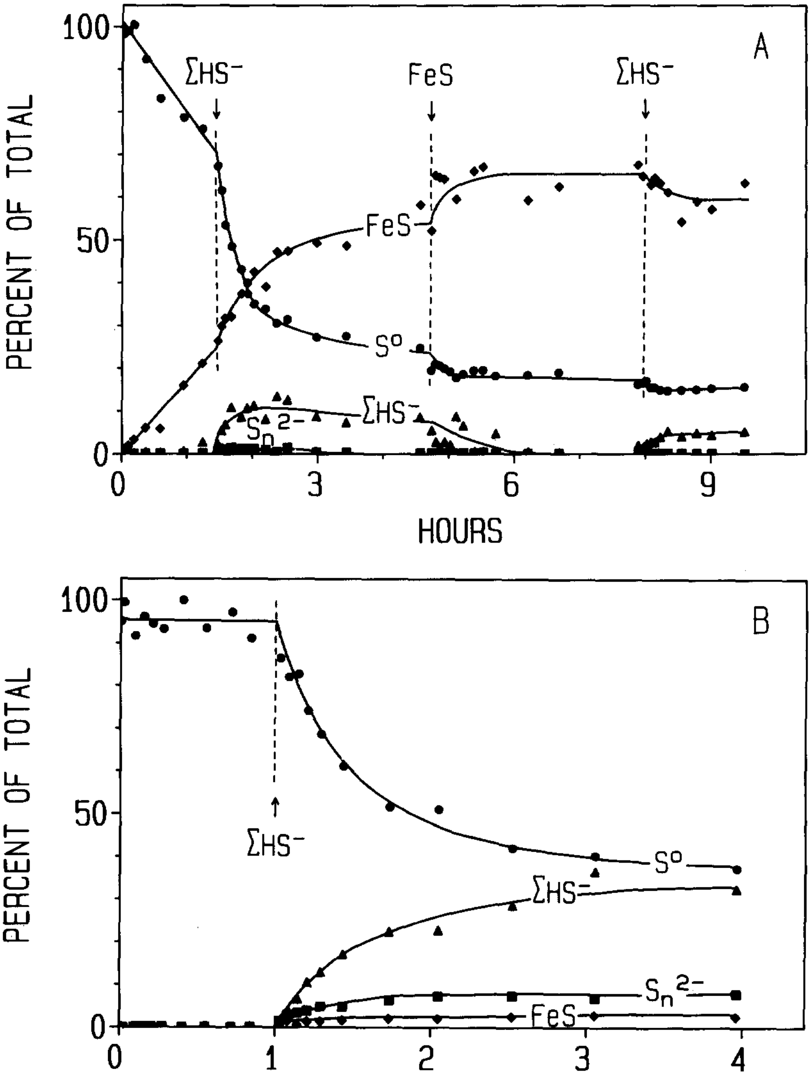

Fig. 4. Time course of isotope exchange with FeS. The radioactivity is expressed in percent of total activity which equals the initial $^{35}S^0$. The arrows point at the times when ΣHS^- and FeS were added. A: Trace amounts of ΣHS^- present at $t = 0$. B: No ΣHS^- present at $t = 0$.

A linear increase in FeS radioactivity accompanied a similar decrease in S^0 radioactivity in Experiment VA (Fig. 4A). The concentration of S^0 did not decrease significantly during this period and thus did not explain the observed change in S^0 radioactivity. We therefore presume that S_n^{2-} and HS^-

were the isotope carriers in the exchange between S^0 and FeS and that $^{35}S_n^{2-}$ and $H^{35}S^-$ formed as described by reaction (3). The ^{35}S-atoms then concurrently exchanged with FeS

$$^{35}S_n^{2-} + n\text{FeS} \rightleftharpoons S_n^{2-} + n\text{Fe}^{35}\text{S} \qquad (13)$$

or

$$H^{35}S^- + \text{FeS} \rightleftharpoons HS^- + \text{Fe}^{35}\text{S} \qquad (14)$$

Polysulfide with S-chain length (n) > 3 is more reactive than HS^- (LaLonde et al. 1987; Luther 1990). Thus, exchange reaction (13) seems to occur more readily than (14).

It was confirmed by the addition of ΣHS^- that S_n^{2-} and possibly HS^- are the isotope carriers, both in experiment VA and VB (Fig. 4A and 4B). The enhanced isotope transport from S^0 to ΣHS^- resulted in an immediate increase in FeS activity and isotopic pseudo-equilibrium was reached within 3 h.

The addition of FeS and the second ΣHS^- addition in experiment VA show the expected changes in ^{35}S distribution (Fig. 4A). More ΣHS^- disturbed the S_n^{2-} equilibrium and less ^{35}S was in S_n^{2-}, concurrently reducing the FeS radioactivity. The 9-fold increase in HS^- concentration in experiment VA after 1.3 h resulted in a fraction exchange for FeS of 0.65. This is about 8-fold higher than observed from the isotope exchange between $\Sigma H^{35}S^-$ and FeS. The isotope that was not exchanged with FeS was distributed between S^0 and ΣHS^-. The isotope content in the ΣHS^- pool was thus about 36% higher than expected from the concentrations of S^0 and ΣHS^-. The formation of S_n^{2-} upon ΣHS^- addition might explain this observation. During the experiment, F(FeS) increased from 0.65 at 4.75 h to 0.83 at 9.5 h.

In experiment VB (Fig. 4B) a fraction exchange for FeS of 0.14 was observed after 4.5 h which shows that pseudo-equilibrium was reached. At pseudo-equilibrium the fraction of ^{35}S that was *not* exchanged with FeS equalled 96%. This ^{35}S was distributed between S^0, S_n^{2-}, and ΣHS^- relative to pool sizes, i.e. $F(S^0) = F(S_n^{2-}) = F(HS^-) = 1$, and verified a complete isotope exchange between these compounds.

Isotope exchange with FeS_2

The experiments done to study ^{35}S exchange between solid phase (S^0 and FeS) had shown that isotope exchange took place only when a soluble sulfur compound, S_n^{2-} or HS^- was present (Fig. 4A and 4B).

We therefore first studied a possible exchange reaction between ΣHS^- and

FeS_2. Two experiments were done, one with $\Sigma H^{35}S^-$ as the labeling agent, the other with $Fe^{35}S_2$. The experiment that was labeled with $\Sigma H^{35}S^-$ revealed a decrease in ΣHS^- concentration of $0.42 \pm 0.15\,\mu mol\,ml^{-1}$ ($n = 5$) during the first 1.5 h (Table 2). The decrease corresponded to a decline in radioactivity of $(100/4.44 \times 0.42)9.5 \pm 3.4\%$ and is equal to the observed decrease in ΣHS^- radioactivity of $5.9 \pm 4.6\%$ (tested by a Student's *t*-test; $p < 0.01$; $f = 8$). Consequently, the observed transfer of ^{35}S from ΣHS^- to FeS_2 does not imply that isotope exchange is responsible for the observed 6.0–8.8% increase in FeS_2 radioactivity, Trace metals on the surface of FeS_2, a typical phenomenon at $pH > 2$ (G. Luther III, pers. com.), will bind HS^- cations and give the apparent exchange between $\Sigma H^{35}S^-$ and FeS_2. No isotope transfer was observed when $Fe^{35}S_2$ was used as the labeling agent (Table 2) and therefore ^{35}S was *not* exchanged between ΣHS^- and FeS_2, i.e. exchange reaction (7a) *did not* proceed

$$H^{35}S^- + FeS_2 \not\rightleftharpoons \quad HS^- + Fe^{35}SS \tag{7b}$$

Pyrite *did not* form when equal concentrations of S^0 and FeS were mixed in experiment VII (reaction (8)) and isotope exchange to FeS_2 was not observed when S^0, ΣHS^-, FeS, and FeS_2 were mixed and labeled with $^{35}S^0$ or $\Sigma H^{35}S^-$ (data not shown). Based on all these observations we therefore conclude that, in contrast to FeS, significant isotope exchange *did not* proceed between FeS_2 and the other reduced sulfur compounds over 24 h.

Isotope exchange and pH

The present study was done in anoxic sterile seawater at pH 7.6 and 20 °C. It is clear that under these conditions isotope exchange is of importance when the biogeochemical sulfur cycle is studied by use of radiolabeled sulfur compounds. However, in natural sediments pH of pore water varies over a broad range, from a minimum of pH 4 in some salt marshes to about pH 8 in some marine sediments.

The kinetics of the isotope exchange is expected to vary with pH. Polysulfide is present as tetrasulfide and pentasulfide at neutral pH and lower (Giggenbach 1972). The S_n^{2-} forms from the reaction between S^0 and HS^- (reaction (2)) or by an incomplete oxidation of ΣHS^- (Chen & Morris 1972; Luther et al. 1986; Millero 1986) and is observed in concentrations $> 300\,\mu M$ in f.ex. salt marsh sediments (Luther et al. 1985). The pK value for S_4^{2-} of 6.3 and 5.7 for S_5^{2-} (Schwarzenbach & Fischer 1960) show that at pH 7 > 90% of the polysulfide is present as S_n^{2-} ions, the most reactive polysulfide form (LaLonde et al. 1987). As pH is lowered, S_n^{2-} becomes

Table 2. Isotope exchange between HS^- and FeS_2. Data from $\Sigma H^{35}S^-$ and $Fe^{35}S_2$ labeled experiments.

Hours	$\Sigma H^{35}S^-$ Labeled				$Fe^{35}S_2$ Labeled			
	Concentration μmol S_{eqv} ml^{-1}		Activity cpm ml^{-1}		Concentration μmol S_{eqv} ml^{-1}		Activity cpm ml^{-1}	
	ΣHS^-	FeS_2	ΣHS^-	FeS_2	ΣHS^-	FeS_2	ΣHS^-	FeS_2
0	4.44 ± 0.10	17.42 ± 0.64	100.0 ± 2.8	0.0 ± 0.0	4.86 ± 0.11	14.12 ± 0.49	0.0 ± 0.0	100.0 ± 1.5
1.5	4.04 ± 0.05	17.39 ± 0.35	94.1 ± 3.6	6.0 ± 0.3	4.38 ± 0.10	14.61 ± 0.56	0.8 ± 0.0	97.6 ± 2.1
24	4.03 ± 0.16	17.06 ± 0.64	91.1 ± 3.1	8.8 ± 0.3	4.43 ± 0.15	13.98 ± 0.81	0.8 ± 0.0	100.8 ± 1.8

Concentrations are expressed in sulfur equivalents (S_{eqv}) and activities are normalized in percent of initial activity. Numbers are the mean of 5 measurements ± s.d. of the mean.

protonated (HS_n^-) and expectedly less reactive resulting in reduced isotope exchange between polysulfide and other sulfur compounds. The same arguments hold for the decrease in reactivity of monosulfide when HS^- becomes protonated (H_2S).

In contrast, FeS and possibly FeS_2 will be more reactive with decreasing pH as their solubility increases. Isotope exchange between $H^{35}S^-$ and FeS is described by the following exchange reactions

$$H^{35}S^- + FeS + H^+ \rightleftharpoons H^{35}S^- + Fe^{2+} + HS^- \rightleftharpoons HS^- + Fe^{35}S + H^+ \tag{4a}$$

$$H^{35}S^- + FeS + H_2O \rightleftharpoons H^{35}S^- + Fe^{2+} + HS^- + OH^- \rightleftharpoons HS^- + Fe^{35}S + H_2O \tag{4b}$$

Reaction (4a) becomes more important at pH below neutral and isotope exchange between HS^- and FeS expectedly increases because of the higher solubility product of FeS at low pH. Temperature also influences chemical reactions as the reaction rate increases with increasing temperature. Thus, isotopic equilibrium is expectedly reached faster at higher temperatures.

At present it is not possible from the theoretical considerations to predict exactly how changes in pH and temperature will influence on S-isotope exchange. For example, at pH lower than 7.6 the expected increase in reactivity of FeS may be balanced by decreased reactivities of poly- and monosulfides. Experiments are now in progress to study pH and temperature effects on ^{35}S exchange.

Conclusions

The present study shows that results from process studies based on tracer experiments labeled with $^{35}S^0$ or $\Sigma H^{35}S^-$ should be interpreted with care. Isotope exchange also influences radiotracer measurements of sulfate reduction rates because of the formation of $^{35}S^0$ which must be included in the analysis together with $AV^{35}S$ and $Fe^{35}S_2$.

Fractionation of the stable sulfur isotopes, ^{32}S and ^{34}S, may result from both unidirectional chemical reactions and equilibrium processes (Tudge & Thode 1950). Bacteria mediate many chemical reactions of importance to the sulfur cycle and may discriminate between the stable sulfur isotopes, ^{32}S and ^{34}S. This bacterial discrimination often leads to a larger enrichment of

^{32}S in the product than does the fractionation from purely chemical reactions (Tudge & Thode 1950). A large variation in the relative proportion of $^{34}S/^{32}S$ is consequently observed in nature (e.g. Goldhaber & Kaplan 1974; Nielsen 1979). Isotopic fractionation data are therefore often used to evaluate the origin of mineral deposits and the bacterial importance for the sulfur cycle (Goldhaber & Kaplan 1980). However, our study implies that isotope exchange to some degree may compensate for the isotope fractionation and weaken the isotopic signal.

Chanton (1985) measured $\delta^{34}S$ isotopic composition of ΣHS^- and FeS in marine sediment down to 75 cm depth and observed significant differences in $\delta^{34}S$ at all depths (= time). Hence, complete isotope exchange was not observed in Chanton's $\delta^{34}S$ measurements in the time scale of years. Isotopic equilibrium between ΣHS^- and FeS probably does not occur under most natural conditions.

We studied isotope exchange between homogeneously distributed sulfur compounds whereas sulfur distribution in natural sediment is much more heterogeneous. Hence, optimal conditions for isotope exchange were established in this study. We have, however, also observed significant isotope exchange between S^0, ΣHS^-, and FeS in marine sediment slurries at pH 7.5 and 20 °C and in undisturbed sediment cores at in situ pH and temperature when these sediments were injected with $^{35}S^0$ or $\Sigma H^{35}S^-$ (Fossing & Jørgensen in prep.).

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