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Isotopic behaviour of sulphate oxygen in the bacterial reduction of sulphate

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Abstract—The oxygen isotopic ratio of the sulphate remaining in the bacterial sulphate reduction has been found to be dependent on the oxygen isotopic ratio of the water in which the sulphate was reduced by bacteria. This finding is interpreted by a mechanism, in which the sulphate exchanges oxygen isotopes with the water through the intermediates in the bacterial reduction of sulphate into sulphide.

INTRODUCTION

In a previous study (MIZUTANI and RAFTER, 1969b), we reported some experimental results showing that sulphate-reducing bacteria preferentially metabolized lighter species, $^{32}S^{16}O_4$, during reduction of sulphate into sulphide, and that the ratio of the ^{18}O enrichment to the ^{34}S enrichment in the sulphate remained was approximately 1 : 4. In this work, we attempt to test the relationship between the ^{18}O enrichment in the remaining sulphate and the oxygen isotopic ratio of the water in which the sulphate is reduced by bacteria, and will describe some possible mechanisms, by which the sulphate exchanges oxygen isotopes with the water through bacterial reduction of sulphate.

EXPERIMENTAL

To see the oxygen isotopic effect of sulphate-reducing bacteria, two series each of purified and mixed culture experiments were run under different conditions. For purified culture experiments the sulphate-reducing bacteria were isolated and purified from the black bottom mud collected from Nagoya harbour, Japan, by repeated subculturing in the medium solution of the following composition: 1 ml of 50% Na-lactate, 0.1 g of dried yeast extract, and 0.1 g of $FeSO_4 \cdot 7H_2O$ in 200 ml of boiled sea water. In mixed culture experiments the black mud itself was used as a bacteria source after all the sulphate in the mud was reduced by bacteria into sulphide. In

order to see whether the oxygen isotopic ratio of the sulphate remaining is affected by a change in the oxygen isotopic ratio of the water in which the sulphate is reduced, each series of culture experiments was duplicated using the medium solution having a different oxygen isotopic ratio of water. As the obtained sulphate-reducing bacteria presumably belong to marine strain, the medium solution was prepared with sea water and some additional nutrients. A known amount of sea water was placed in a beaker, and then evaporated to dryness on a water bath. The salt thus obtained was dissolved and diluted with the waters of different oxygen isotopic ratio to the same concentration with the sea water. To this salt solution additional nutrients such as Na-lactate and dried yeast extract were added to accelerate bacterial growth. In purified culture experiments $FeSO_4 \cdot 7H_2O$ was also added to the medium solution to remove the evolved H_2S as ferrous sulphide, because the excess H_2S resulted from the reduction of sulphate poisons the medium solution. The chemical composition of medium solution used in the isotopic fractionation experiments is shown in Table 1.

Table 1. Composition of medium solution for isotopic fractionation experiments

Purified culture experiment	
Exp. 1	10ml of 50% Na-lactate, 2.5g of $FeSO_4 \cdot 7H_2O$, and 0.5g of dried yeast extract in 1ℓ of sea-salt solution equivalent to sea water in salt concentration.
Exp. 2	10ml of 50% Na-lactate and 2.5g of $FeSO_4 \cdot 7H_2O$, in 1ℓ of the sea salt solution.
Mixed culture experiment	
Exp. 3	6ml of 50% Na-lactate in 1ℓ of the sea-salt solution.
Exp. 4	Sea-salt solution.

The isotopic fractionation experiments were conducted in the similar manner to that described previously (MIZUTANI and RAFTER, 1969b). One hundred fifty ml of medium solution was placed into 250ml flask with inlet and outlet tubes inserted through a rubber bung. To produce an anaerobic condition, oxygen free nitrogen gas was swept through this flask for 15min. The flask was then inoculated with the sulphate-reducing bacteria, and bubbled with nitrogen gas for about 15min again. In mixed culture experiments 30g of black mud was added into the flask as a bacteria source instead of inoculation. The flask was closed, covered with thin aluminium foil, and placed in a temperature-controlled water bath. After 2 ~ 89 days the flask was removed from the water bath. The excess H_2S was precipitated as zinc sulphide by adding $Zn(CH_3COO)_2 \cdot 2H_2O$ to the solution. The remaining sulphate was then recovered as barium sulphate by the usual method. The isotopic analyses were performed using the methods described by RAFTER (1967) and MIZUTANI (1971). About half the isotopic analyses were made by RAFTER at Institute of Nuclear Science, Lower

Hutt, New Zealand, and the rest by MIZUTANI at Nagoya University, Nagoya, Japan. All analyses were duplicated, and averaged results were given in the delta notation:

$$\delta^{18}O = [\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{SMOW}} - 1] \times 1{,}000 \ (‰)$$

$$\delta^{34}S = [\frac{(^{34}S/^{32}S)_{sample}}{(^{34}S/^{32}S)_{met.\ S}} - 1] \times 1{,}000 \ (‰)$$

In order to see whether the reduced sulphur compounds are oxidized during the recovery process for the remaining sulphate, the recovery of sulphate was duplicated in two instances of purified culture experiments, using waters of $\delta^{18}O = +27.5$ and $-28.7‰$ for dilution and washing. In either instance, the sulphate recovered revealed a difference of $0.4 \sim 0.6‰$ in $\delta^{18}O$ value with the $\delta^{18}O$ value of water, as shown in Table 2 (Exp.1-b-2 and 1-b-4). This difference is obviously due to the incorporation of water oxygen into the sulphate which was formed by the oxidation of reduced sulphur compounds during the recovery process. In the other instances of our experiments, distilled water having a $\delta^{18}O$ value of $-7‰$ was used for dilution and washing. The error from such effect may be smaller than the error of mass spectrometer measurement of $0.2 \sim 0.3‰$.

Table 2. Bacterial fractionation experiments

Purified culture experiment

Sample No.	Temp. °C	Time days	g/ℓ	F	$SO_4^{=}$ $\delta^{18}O$,‰	$\delta^{34}S$,‰	H_2O $\delta^{18}O$,‰
Exp. 1-a-1	25	0.0	3.48	1.00	10.0	14.9	26.2
-a-2	"	1.9	2.92	0.84	10.2	16.1	25.7
-a-3	"	6.9	0.98	0.28	14.4	25.2	26.1
-a-4	"	10.9	1.00	0.29	14.5	25.4	25.7
-b-1	25	0.0	3.42	1.00	9.9	15.6	1.5
-b-2	"	3.1	1.67	0.49	10.7* 10.1**	20.7	1.3
-b-3	"	6.2	1.21	0.35	11.4	24.7	1.4
-b-4	"	9.2	1.14	0.33	11.3* 10.9**	24.2	1.5
-c-1	25	0.0	3.37	1.00	10.0	15.3	−25.8
-c-2	"	1.9	2.32	0.69	9.4	17.3	−25.7
-c-3	"	6.9	0.92	0.27	8.6	28.7	−25.7
-c-4	"	10.9	0.96	0.29	8.7	28.6	−25.7
Exp. 2-a-1	25	0.0	3.40	1.00	10.3	15.6	11.7
-a-2	"	13.2	2.89	0.85	11.0	17.3	11.7
-a-3	"	20.1	2.46	0.72	10.7	17.6	11.1
-a-4	"	34.9	1.90	0.56	11.8	21.3	12.2
-a-5	"	52.0	1.74	0.51	11.7	21.7	–

Continued on the next page.

Mixed culture experiment

Sample No.	Temp. °C	Time days	$SO_4^{=}$ g/ℓ	F	$\delta^{18}O$,‰	$\delta^{34}S$,‰	H_2O $\delta^{18}O$,‰
Exp. 3-a-1	30	0.0	2.27	1.00	9.9	19.5	19.0
-a-2	〃	2.7	1.85	0.81	12.4	22.8	19.7
-a-3	〃	3.8	1.54	0.67	14.6	26.8	–
-a-4	〃	5.9	0.53	0.23	20.0	37.4	19.5
-a-5	〃	6.8	0.21	0.09	22.6	42.3	–
-b-1	30	0.0	2.27	1.00	9.7	19.3	−12.7
-b-2	〃	3.7	1.52	0.67	10.6	26.1	−13.7
-b-3	〃	5.9	0.61	0.27	11.8	37.2	−12.6
-b-4	〃	6.8	0.32	0.14	16.4	41.0	−12.7
-b-5	〃	6.8	0.26	0.11	12.4	43.2	−13.4
-b-6	〃	7.7	0.15	0.07	15.1	43.6	–
	〃						
Exp. 4-a-1	30	0.0	2.27	1.00	9.4	20.2	–
-a-2	〃	5.1	2.17	0.96	10.6	21.0	− 0.1
-a-3	〃	13.9	2.04	0.90	11.7	22.1	− 0.8
-a-4	〃	28.9	1.98	0.87	12.6	24.8	0.2
-a-5	〃	62.9	1.81	0.80	15.2	28.1	–
-a-6		89.1	1.75	0.77	16.3	29.0	0.1
-b-1	30	0.0	2.27	1.00	9.4	19.7	− 7.8
-b-2	〃	7.0	2.09	0.92	10.0	21.9	− 6.7
-b-3	〃	15.0	2.01	0.88	10.6	23.0	− 6.5
-b-4	〃	30.0	1.93	0.85	11.8	25.2	− 7.2
-b-5	〃	56.1	1.72	0.76	12.6	29.8	− 7.4
-b-6	〃	87.0	1.57	0.69	13.7	33.1	− 6.5

* Recovered using water of $\delta^{18}O$ = +27.5‰ for dilution and washing.
** Recovered using water of $\delta^{18}O$ = −28.7 ‰ for dilution and washing.

Results and Discussion

Table 2 shows the results obtained in this work. It has been known that the results of bacterial sulphate reduction in a closed system can be described by the following linear equation.

$$\delta - \delta_0 = 1{,}000\ (\alpha - 1)\ \ln F$$

where δ_0 is δ value of the original sulphate, δ is δ value of sulphate when the fractionation of sulphate remaining is F, and α is the fractionation factor. When the isotopic fractionation data obtained in this work were plotted against natural logarithms of the fraction of remaining sulphate, there is a good straight line relationship in each series of experiments, except Experiment 3, as shown in Figs.1 ~ 4. The slope of curve indicates the value of 1,000 ($\alpha - 1$), from which the fractionation factor can be estimated. For Experiment 3 (Fig.3) the α values have been estimated from the isotopic fractionations before 60% reduction, as the slope of curve decreases after 60% reduction. A change in the α value for $\delta^{34}S$ also exists to a certain degree in Experiments 1 and 2 (Figs.1 and 2, respectively) at a higher percent reduction. Changes

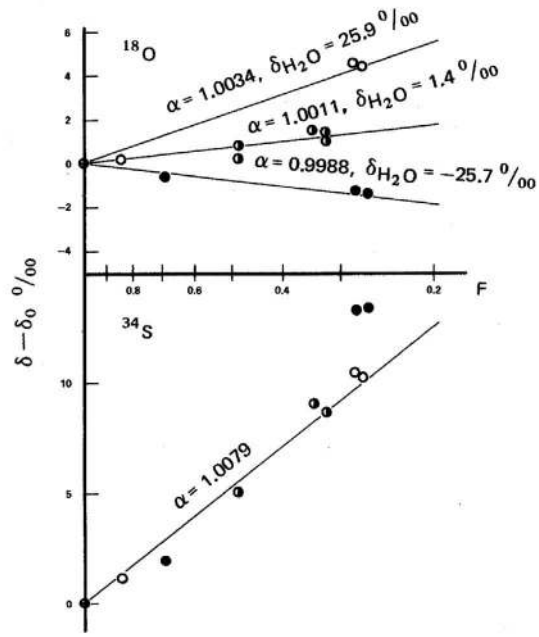

Fig.1. Oxygen-18 and sulphur-34 fractionations *vs.* fraction of remaining sulphate in Exp.1. ⊕, Original sulphate; ○, ◑, and ●, Exps. 1-a, 1-b, and 1-c, respectively; α, fractionation factor and δ_{H_2O}, $\delta^{18}O$ value of water.

of this sort may be due to the change in the mechanism of sulphate reduction which is presumably caused by the accumulation of fermentation products.

Comparison of the results of duplicate experiments indicates that the α value for sulphate oxygen changes with the $\delta^{18}O$ value of water, while the α value for sulphate sulphur does not change. This evidence suggests that the sulphate exchanges oxygen isotopes with the water in which the sulphate is reduced by bacteria. However, it has been known for some time that the rate of oxygen isotopic exchange reaction between sulphate ions and water is very slow in neutral and alkaline solutions. For example in a solution of pH = 7.0, the half-time of exchange reaction is about 2,000 y at 25°C (LLOYD, 1968). Under our experimental conditions, sulphate is unlikely to exchange oxygen isotopes directly with water. Therefore, the observed oxygen isotopic fractionation may be explained as a result of bacterial sulphate reduction, in which the intermediates in the reduction of sulphate into sulphide must have played an important role in exchanging oxygen isotopes between sulphate and water.

According to HARRISON and THODE (1958), the bacterial sulphate reduction process follows the following consecutive steps:

$$SO_4^= + \text{enzyme} \xrightarrow{I} SO_4^= \text{- enzyme complex} \xrightarrow{II} SO_3^= \xrightarrow{III} H_2S$$

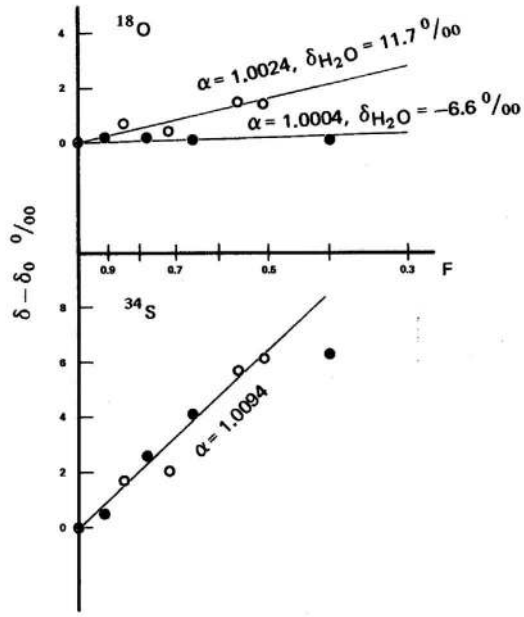

Fig.2. Oxygen-18 and sulphur-34 fractionations *vs.* fraction of remaining sulphate in Exp.2. ⊕, Original sulphate; ○ and ●, Exps. 2-a and 2-b, respectively; α, fractionation factor; and δ_{H_2O}, $\delta^{18}O$ value of water.

If Step I is rate-controlling, there will be small oxygen and sulphur isotopic effects because Step I does not involve the breaking of a S-O bond. However, when Step I is a rapid equilibrium process followed by a relatively slow reaction for Step II, there will be a possibility of exchanging sulphate oxygen with water oxygen through the $SO_4^=$ enzyme complex, provided that the sulphate oxygen of the complex can be exchanged with water oxygen at a relatively rapid rate (Step IV).

$$SO_4^= + \text{enzyme} \overset{I}{\rightleftharpoons} SO_4^=\text{-enzyme complex} \overset{II}{\rightarrow} SO_3^= \overset{III}{\rightarrow} H_2S$$
$$\Vert\ IV$$
$$H_2O$$

For the oxygen isotopic exchange reaction between sulphate ions and water, the equilibrium fractionation factor has been estimated to be 1.029 at 25°C by extrapolation from the experimental results of the present authors (MIZUTANI and RAFTER, 1969a). A trend towards this ^{18}O fractionation may be expected in the oxygen isotopic exchange reaction between sulphate ions and water through the $SO_4^=$-enzyme complex. Since Step II is rate-controlling in the above reduction process and involves the breaking of a S-O bond, there will be a large kinetic isotope effect in Step II, in which

$^{32}S^{16}O_4^=$-enzyme complex reacts faster. For example in sulphur isotopic fractionation, a maximum kinetic isotope effect of 1.022 (α value) will be expected from Step II, as described by HARRISON and THODE (1958). In oxygen isotopic fractionation the kinetic isotope effect in Step II will be superimposed upon the equilibrium isotope effect between sulphate and water through Steps I and IV.

Another possible pathway of exchanging sulphate oxygen with water oxygen is as follows:

$$SO_4^= + \text{enzyme} \overset{I}{\rightleftharpoons} SO_4^=\text{-enzyme complex} \overset{II}{\rightleftharpoons} SO_3^= \overset{III}{\rightarrow} H_2S$$
$$\Downarrow V$$
$$H_2O$$

KEMP and THODE (1968) suggested that an equilibrium isotope effect is possible between sulphate and sulphite sulphurs when Steps I and II are rapid equilibrium processes followed by a relatively slow reduction of sulphite (Step III), and that a sulphur isotopic effect of 1.049 (α value) may be expected from the superimposition of the equilibrium isotope effect between sulphate and sulphite (α = 1.024, 25° C upon the kinetic isotope effect (α = 1.025) involved in the reduction of sulphite. If

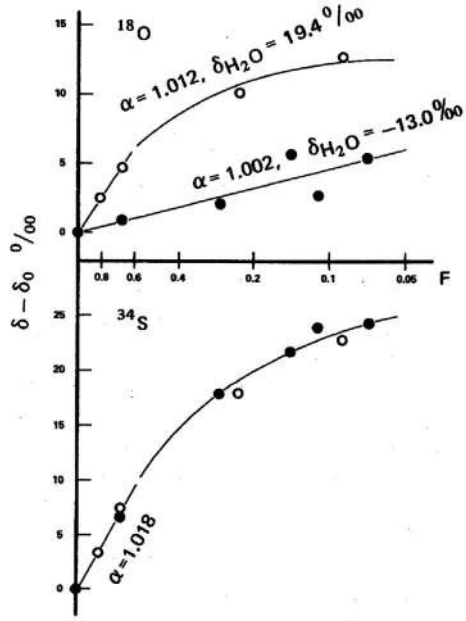

Fig.3. Oxygen-18 and sulphur-34 fractionations *vs.* fraction of remaining sulphate in Exp.3. ⊕, Original sulphate; ○ and ●, Exps. 3-a and 3-b, respectively; α, fractionation factor; and δ_{H_2O}, $\delta^{18}O$ value of water.

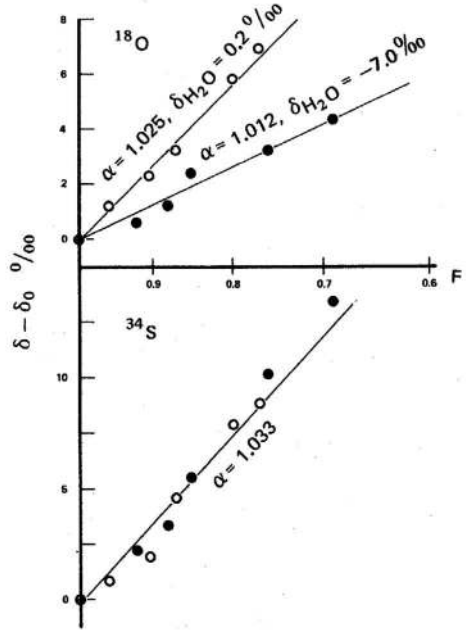

Fig.4. Oxygen-18 and sulphur-34 fractionations *vs.* fraction of remaining sulphate in Exp.4. ⊕, Original sulphate; ○ and ●, Exps. 4-a and 4-b, respectively; α, fractionation factor; and δ_{H_2O}, $\delta^{18}O$ value of water.

this is the case an equilibrium isotope effect may be also expected between sulphate and water oxygens through Steps I, II and V because sulphite exchanges oxygen isotope with water at a very rapid rate (LLOYD, 1968), and the equilibrium isotope effect between sulphite and water will be superimposed upon the kinetic isotope effect in Step III.

It is interesting that a value of sulphur isotopic fractionation factor as large as 1.033 has been obtained from Experiment 4, in which sulphate was reduced under more natural condition than in the other Experiments. The sulphur isotopic fractionation factor of 1.033 is much larger than that expected from the kinetic isotope effect in Step II or Step III. This suggests the possibilities that in Experiment 4 the superimposition of isotopic effects suggested by KEMP and THODE (1968) may have existed to a certain degree, and that in this Experiment the reflection of the difference in the $\delta^{18}O$ value of water on the oxygen fractionation factor may be therefore due to the equilibrium isotope effect between sulphate and water through Steps I, II and V as well as Steps I and IV. Such condition, however, would not have been likely in the other Experiments, as the α values obtained for sulphur isotopic fractionation are small enough to be explained by the kinetic isotope effect in Step II. In Experiments

1, 2, and 3 the reflection of difference in the $\delta^{18}O$ value of water on the oxygen fractionation factor is presumably due to the equilibrium isotope effect between sulphate and water through Steps I and IV.

Conclusion

(1) The oxygen isotopic ratio of the sulphate remaining in the bacterial reduction of sulphate depends upon that of the water in which the sulphate is reduced.

(2) It is likely that the oxygen isotopic exchange between sulphate ions and water proceeds through the intermediates in the bacterial reduction of sulphate into sulphide.

(3) The ratio of the ^{18}O enrichment to the ^{34}S enrichment in the sulphate remaining is changeable, depending upon the oxygen isotopic ratio of the water, in which the sulphate is reduced, and presumably upon the mechanism of bacterial reduction of sulphate.

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