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研究課題名（和文）Determining sulfur isotope fractionation values of individual enzymes and how they evolve through time.

研究課題名（英文）Determining sulfur isotope fractionation values of individual enzymes and how they evolve through time.

研究代表者
ＭｃＧｌｙｎｎ　Ｓｈａｗｎ・Ｅ.　(McGlynn, Shawn)

東京工業大学・地球生命研究所・准教授

研究者番号：１０７５１０８４

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研究成果の概要（和文）：地球上で最も古い生命の記録は化学的なものである。生物は原子を固有の比率で使用するからである。生物学的な同位体比を示す元素の一つが硫黄である。本研究では、太古の地球上にどのような生物が存在し、硫黄同位体比に痕跡を残したのかに注目した。その結果、古代の硫黄同位体比の大部分をAPSレダクターゼと呼ばれる1つの酵素（化学反応を非生物的な反応よりも迅速に起こす生物学的な触媒）が担っていることが明らかになった。さらに、酵素反応の前進速度と後退速度が利用可能なエネルギー量に依存することから、古代生物の栄養状態を理解できることもわかった。本研究は、生化学や生命と古代地球を統合するものである。

研究成果の学術的意義や社会的意義
We found that the evolutionary history of sulfate respiration is complex, but probably not among the most ancient metabolisms on Earth. We determined that a single enzyme in the respiratory pathway appears to control the whole cell rate under certain conditions.

研究成果の概要（英文）：The oldest record of life on Earth is in the form of non-equilibrium isotope ratios, because biology uses isotopes in unique compared to equilibrium (abiotic) chemistry. One element which shows biological like isotope ratios deep in time is sulfur. In this research, we focused on which type of organisms may have been on the ancient Earth and left signals in sulfur isotope ratios. We found that the enzyme APS reductase (a biological catalyst which allows chemistry to occur more quickly than non-biological reactions) may be responsible for a substantial portion of the ancient sulfur ratios. Furthermore, we found that we could understand the nutritional state of ancient organisms, because the forward and reverse rates of the enzymatic reaction depends on the amount of energy available. Our work integrates biochemistry and life with knowledge of the ancient Earth.

研究分野：Geobiology

キーワード：enzyme mechanism　enzyme evolution　kinetic isotope effect　sulfate reduction　geobiology　microbial evolution　Earth-Life Science　geochemistry

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１．研究開始当初の背景 (Background at the beginning of the research)

(1) Geochemists have documented extensive variations mass dependent sulfur isotope fractionation. In certain cases, these variations suggest biological sulfate reduction, which leaves sulfide as a product preserved in the sedimentary record containing an isotope ratio distinct from the equilibrium value. Which organisms, and the type of physiology responsible for this record, remains unclear.

(2) A Bio-isotopic model accounting for sulfur isotope fractionation during biological sulfate reduction was published in 2014 by Wing and Halevy. This wasa major advance, since it related the kinetics and thermodynamics of metabolism, to the kinetic and equilibrium isotope fractionation factors of different reactions. However, kinetic isotope fractionation factors for the individual enzymes was unknown. Furthermore, it was unknown if the fractionation factors might change during evolution.

２．研究の目的 (Purpose of the research)

The purpose of the research was to discover the kinetic isotope fractionation for enzymes involved in respiratory sulfate reduction, and if it changes evolutionarily.
The objectives were:
Objective 1) To conduct evolutionary analyses of the protein families involved in MSR.
Objective 2) Purification of individual enzymes involved in sulfate reduction.
Objective 3) To determine fractionation values for sulfate reducing enzymes by conducting compound specific sulfur isotope measurements of enzyme substrates and products.

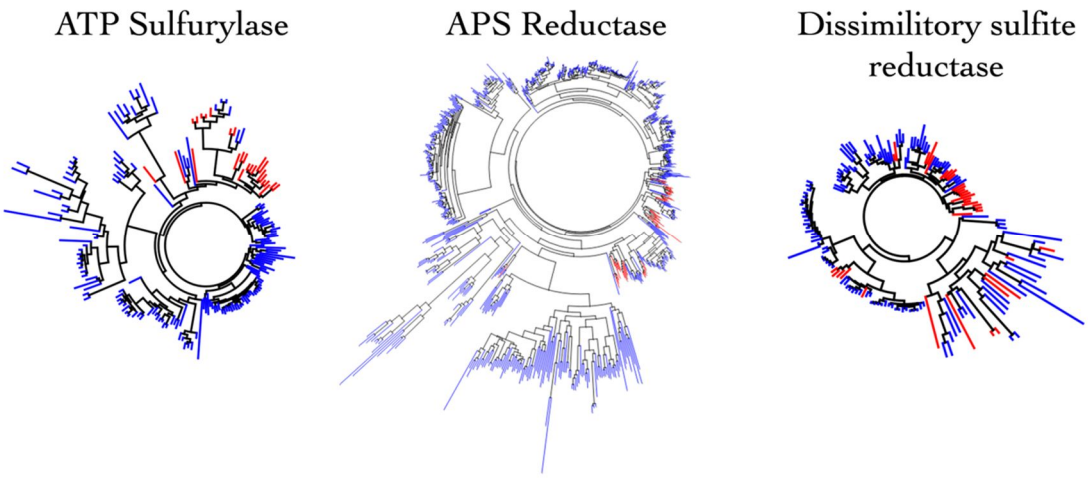
３．研究の方法 (Research methods)

(1) We used the genome taxonomy database for sequence collection, and current alignment and tree building protocols for our evolutionary analyses. In addition to assessing similarity with blast score cutoffs, we also consulted the COG data base
(2) We took two approaches for obtaining the enzymes.
-First, we obtained them from a host organism: *Desulfovibrio vulgaris* Miyazaki. These enzymes form the foundation for which to assess the validity of heterologous expression.
- Second, we expressed enzymes in *E.coli*, and also in a cell free expression system (PURE-Frex). We evaluated the expression of these different proteins from these preparations.
Isotopes were measured with a Neptune instrument in our collaborator's lab at Caltech.

４．研究成果 (Research results)

Objective 1) To conduct evolutionary analyses of the protein families involved in MSR.
We constructed phylogenetic trees for the enzymes involved in sulfate reduction as shown below. In addition, we constructed phylogenetic trees for all enzymes available in the Conserved Orthologous Groups (COGS) database (Berkemer and McGlynn 2020 MBE; Figure 1). We analyzed two components of the protein phylogeny to assess antiquity: first, the separation of archaeal and bacterial domains. Second, we analyzed the branch length distances between the domains in the tree. The second part is motivated by the idea that long branch lengths between the domains may indicate presence in the last common ancestor (LUCA), followed by evolution into the archaeal and bacterial domains. We found in the case of enzymes involved in sulfate reduction, they all display evidence of interdomain lateral gene transfer. Second, we found that the branch lengths between the domains is not long relative to intra-domain branch lengths. These two lines of evidence suggest that these proteins were not in the LUCA.

Figure 1. Protein phylogenies for the three enzymes involved in dissimilatory sulfate reduction. Sequences found in archaea are red, and blue corresponds to bacteria. The results were published in Berkemer and McGlynn, MBE, 2020.

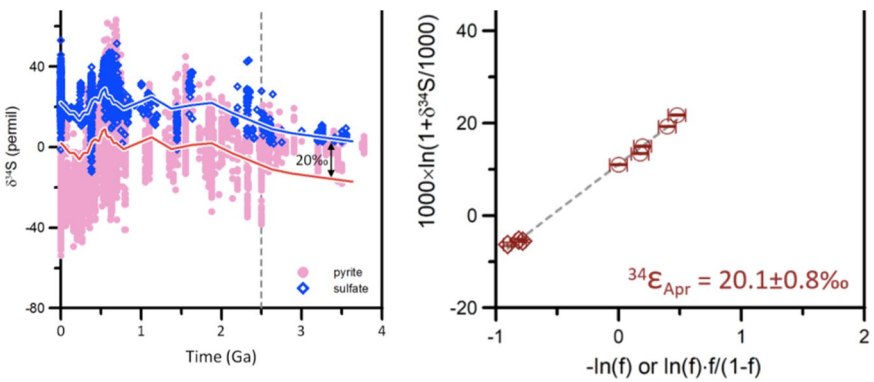


Objective 2) Purification of individual enzymes involved in sulfate reduction. We obtained the APS reductase (APS-R) and dissimilatory sulfite reductase (DSR) proteins from *Desulfovibrio vulgaris* Miyazaki, and we assayed the APS-R, determining the apparent kinetic isotope fractionation factor of the enzyme for the first time.

We also extensively tested heterologous expression of APS-R constructs in *E.coli*. Our basic result, and across conditions tested, was that the protein was not suitable for heterologous expression in our laboratory. Therefore, we turned to a cell-free expression system. We found that the constructs could be expressed in this way, however we observed currently unexplained cleavage of the protein during purification, and we have not been able to solve this very unique problem.

Objective 3) To determine fractionation values for sulfate reducing enzymes by conducting compound specific sulfur isotope measurements of enzyme substrates and products.
We assayed the APS-R, determining the apparent kinetic isotope fractionation effect (KIE) of the enzyme for the first time. We found a correspondence between

Figure 2 Showing the change in sulfur isotopes between pyrite and sulfate through time on the left, and the apparent kinetic isotope fractionation factor of the APS reductase enzyme on the right. The ancient sulfur isotope fractionation value matches the enzyme value, implying that the cells which left this signal behind were operating under thermodynamically driven conditions, which would allow the expression of the kinetic isotope value. The results were published in Nature Communications (Sim et al 2019).



the apparent enzyme KIE, and the earliest sulfur isotope fractionation values on Earth (both are about 20 per mil; Figure 2). Furthermore, we integrated this result into the bio-isotopic metabolic model developed by Wing and Halevy. Our results were consistent with the most ancient cells having been thermodynamically strongly driven, whereas our results were consistent with sulfate reducers becoming more metabolically non-competitive (and therefore less thermodynamically driven) after the great oxygenation event of Earth. The work integrated biochemistry, the sedimentary isotope record, and knowledge of microbial physiology through time.

Future work: Although we were able to measure the KIE value of the APS-R enzyme, and more fully understand the evolution of the 3 enzymes involved in microbial sulfate respiration, more work is needed to address the question of KIE values varying over evolutionary time. We hope to pursue this research more in the future.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　5件）

１．著者名 Wang Po-Hsiang、Fujishima Kosuke、Berhanu Samuel、Kuruma Yutetsu、Jia Tony Z.、Khusnutdinova Anna N.、Yakunin Alexander F.、McGlynn Shawn E.	４．巻 9
２．論文標題 A Bifunctional Polyphosphate Kinase Driving the Regeneration of Nucleoside Triphosphate and Reconstituted Cell-Free Protein Synthesis	５．発行年 2019年
３．雑誌名 ACS Synthetic Biology	６．最初と最後の頁 36～42
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acssynbio.9b00456	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Sim Min Sub、Ogata Hideaki、Lubitz Wolfgang、Adkins Jess F.、Sessions Alex L.、Orphan Victoria J.、McGlynn Shawn E.	４．巻 10
２．論文標題 Role of APS reductase in biogeochemical sulfur isotope fractionation	５．発行年 2019年
３．雑誌名 Nature Communications	６．最初と最後の頁 1-9
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-018-07878-4	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 McGlynn Shawn E.、Glass Jennifer B.、Johnson-Finn Kristin、Klein Frieder、Sanden Sebastian A.、Schrenk Matthew O.、Ueno Yuichiro、Vitale-Brovarone Alberto	４．巻 105
２．論文標題 Hydrogenation reactions of carbon on Earth: Linking methane, margarine, and life	５．発行年 2020年
３．雑誌名 American Mineralogist	６．最初と最後の頁 599～608
掲載論文のDOI（デジタルオブジェクト識別子） 10.2138/am-2020-6928CCBYNCND	査読の有無 有
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１．著者名 Berkemer Sarah J、McGlynn Shawn E	４．巻 37
２．論文標題 A New Analysis of Archaea-Bacteria Domain Separation: Variable Phylogenetic Distance and the Tempo of Early Evolution	５．発行年 2020年
３．雑誌名 Molecular Biology and Evolution	６．最初と最後の頁 2332～2340
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/molbev/msaa089	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Linard Benjamin、Ebersberger Ingo、McGlynn Shawn E、Glover Natasha、Mochizuki Tomohiro、Patricio Mateus、Lecompte Odile、Nevers Yannis、Thomas Paul D、Gabaldon Toni、Sonnhammer Erik、Dessimoz Christophe、Uchiyama Ikuo	4．巻 in press
2．論文標題 Ten Years of Collaborative Progress in the Quest for Orthologs	5．発行年 2021年
3．雑誌名 Molecular Biology and Evolution	6．最初と最後の頁 in press
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/molbev/msab098	査読の有無 有
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1．著者名 Gastoldi Lucia、Ward Lewis M.、Nakagawa Mayuko、Giordano Mario、McGlynn Shawn E.	4．巻 36
2．論文標題 Changes in ATP Sulfurylase Activity in Response to Altered Cyanobacteria Growth Conditions	5．発行年 2021年
3．雑誌名 Microbes and Environments	6．最初と最後の頁 n/a～n/a
掲載論文のDOI（デジタルオブジェクト識別子） 10.1264/jsme2.ME20145	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計7件（うち招待講演　7件／うち国際学会　4件）

1．発表者名 Shawn McGlynn
2．発表標題 What role does metal availability play in evolution of life on the planet?
3．学会等名 Gordon Research Conference Geobiology（招待講演）（国際学会）
4．発表年 2020年

1．発表者名 Shawn McGlynn
2．発表標題 Linking Enzyme Evolution to the Rock Record with Stable Isotope Enabled Enzymology
3．学会等名 Deep Carbon Observatory（招待講演）（国際学会）
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Shawn McGlynn

２．発表標題
New insights into microbial iron cycling at a marine ferrous carbonate hot spring

３．学会等名
Marine Biotechnology Conference（招待講演）（国際学会）

４．発表年
2019年

１．発表者名
Shawn McGlynn

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Relating energy, isotopes, and cell physiology

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JSME The conference of the Japanese Society of Microbiology（招待講演）

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2019年

１．発表者名
Shawn McGlynn

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Energy Controlled Distribution of Isotopes in Biocatalytic Systems: Case Study on Adenylyl-Sulfate Reductase

３．学会等名
Helmholtz-Zentrum Umweltforschung（招待講演）

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2018年

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Shawn McGlynn

２．発表標題
Energy Controlled Distribution of Isotopes in Biocatalytic Systems: Case Study on APS Reductase.

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４．発表年
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Shawn McGlynn

２．発表標題
Energy Controlled Distribution of Isotopes in Biocatalytic Systems: Case Study on APS Reductase

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４．発表年
2018年

〔図書〕　計1件

１．著者名	４．発行年
Shawn E. McGlynn	2021年
２．出版社	５．総ページ数
De Gruyter	59-80
３．書名	
Biological Isotope Fractionation and Earth History: From Enzymes, to cells, to ecosystems. In: Metals, Microbes, and Minerals - The Biogeochemical Side of Life Ed. Peter M. H. Kroneck and Martha E. Sosa Torres	

〔産業財産権〕

〔その他〕

https://sites.google.com/elsi.jp/mcglynn/

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	上野　雄一郎 (Ueno Yuichiro) (90422542)	東京工業大学・理学院・教授 (12608)	

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関