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研究課題名（和文）Intramolecular isotope analysis of lipids as a novel biological and environmental proxy

研究課題名（英文）Intramolecular isotope analysis of lipids as a novel biological and environmental proxy

研究代表者

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研究成果の概要（和文）：代謝は複雑なシステムであり、炭素同位体（13Cと12C）、またはそれらの比率は、生物のフラックスと生理学的状態を記録することができます。 したがって、有機分子の安定同位体組成は、多くの分野で重要です。 特に脂質は、堆積性有機物として非常に長期間保存されるため、興味深いターゲットとなります。
このプロジェクトでは、植物の脂肪酸の各位置の13C/12C同位体組成を測定することができました。 したがって、情報量は、すべての位置の平均同位体組成のみを提供する現在の方法と比較して増加します。 したがって、細胞内の同位体分別に関する前例のない詳細を提供します。

研究成果の学術的意義や社会的意義
The results have provided detailed information on how stable isotopes (13C/12C) behave inside a cell. We can use this understanding to interpret and predict future data obtained from lipids, either directly from living organisms or from organic matter from rocks.

研究成果の概要（英文）：Metabolism is a complex system, and carbon isotopes (13C and 12C), or the ratio of them, can record the fluxes and physiological status of an organism. The stable isotope composition of organic molecules is thus of importance in many disciplines. Lipids in particular, provide an interesting target since they are preserved in very long terms as sedimentary organic matter.
In this project, we have developed a method to measure the isotope composition of lipids in a way that provides unprecedented information. Specifically, we were able to measure the 13C/12C isotope composition of each position in fatty acids from plants. The amount of information is thus increased compared with current methods that provide only the average isotope composition of all positions. We thus provide unprecedented details regarding isotope fractionation within a cell.

研究分野：Biogeochemistry

キーワード：Lipids　Isotope　Biomarker

１．研究開始当初の背景

Stable isotope of light elements (C, H, O, N, S) are employed to trace biogeochemical cycles in present and past Earth environments. The isotopic composition of biomolecules is directly linked to their source, the pathway and fluxes associated with their biosynthesis and external factors such as temperature or the CO_2 pressure. Thus, the isotopic composition records the conditions of their biosynthesis. Lipids are an important class of biomarkers as they are preserved in sediments under different chemical forms, (free lipids, bitumen, kerogen), and their isotope composition can be an important source of information.

Thus far, most $\delta^{13}C$ measurements of organic molecules are made after their conversion to CO_2 which allows the precision required to observe natural abundance variations. The conversion step leads to a $\delta^{13}C$ value which is the average of $\delta^{13}C$ values for all positions in the molecule. The measurement of δ values of given positions within a molecule, called position-specific isotope analysis (PSIA), is only accessible via specific methods (Gilbert, 2021): chemical or enzymatic degradation of specific C-atom positions and subsequent $\delta^{13}C$ determination (Monson and Hayes, 1980; Rossmann et al., 1991), in-source fragmentation in a mass spectrometer (Eiler et al., 2013; Neubauer et al., 2018), thermal breakdown coupled to IRMS analysis (“on-line pyrolysis”) (Corso and Brenna, 1997; Gilbert et al., 2016) and direct analysis by nuclear magnetic resonance (Jézéquel et al., 2017). While the potential of position-specific isotope analysis is tremendous, its application to lipids, despite their important in biogeochemistry, has never been exploited.

２．研究の目的

The goal of the project is to measure 13C-PSIA of lipids of different organisms and to decipher the determinants of $^{13}C/^{12}C$ ratios at each position in the carbon chain of fatty acids. PSIA can be a useful tool to trace past environmental conditions and metabolism or ecosystem evolution throughout Earth history.

３．研究の方法

In this project, we use isotopic ^{13}C NMR to measure the position-specific isotope composition of fatty acids from coconut and sunflower oils. The choice of using vegetable oils is obvious when considering the amount necessary for isotopic NMR measurements, namely, few hundred milligrams of pure compound. In addition, the advantage of studying fatty acids biosynthesis is that the commitment of acetyl-CoA to lipids is around 100% in plant seeds (Schwender et al., 2006; Alonso et al., 2007; Paula Alonso et al., 2010), making it a very simple system. Isotopic ^{13}C NMR allows the measurement of 6 C-atom positions within the carbon chain of fatty acids. In addition, we use a chemical method to break the double bond of unsaturated fatty acids and have access to 12 C-atoms, providing further insights into the origin of the isotope fractionation in fatty acids (Fig. 1).

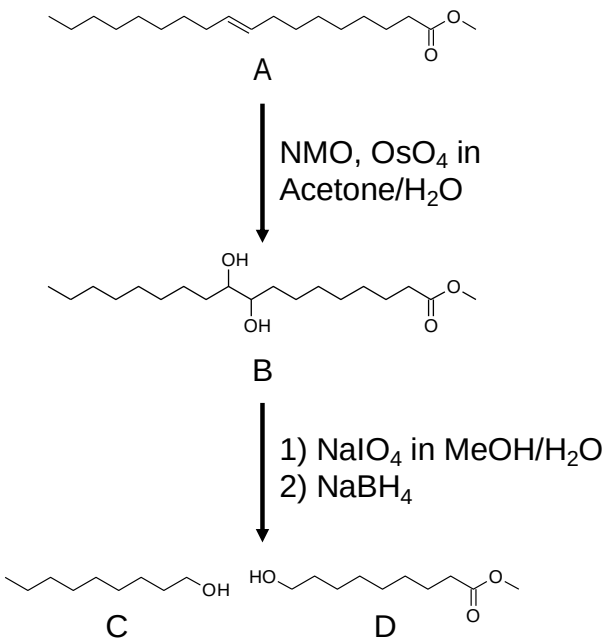

Figure 1. Chemical cleavage of methyl oleate that we used in our study

４．研究成果

The results are presented in Fig. 2. Clearly, there is an alternation between odd and even positions: the odd positions being systematically ^{13}C-enriched compared with even positions. However, the only exception is the two last positions (C_{n-1} and C_n) exhibit the same $\delta^{13}C$ values. Thus, the measured ^{13}C patterns are not totally in accordance with the conventional view of the relative ^{13}C-depletion of acetogenic lipids and their alternation of ^{13}C-enriched and ^{13}C-depleted carbon positions. The results presented here provide a new evaluation of the isotopic fractionation associated with fatty acids biosynthesis. Whereas it is commonly admitted that the pyruvate dehydrogenase (PDH) is responsible for the ^{13}C distribution within fatty acids, data from the present work demonstrate that the conversion of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACC) needs to be considered while explaining the measured non-stochastic ^{13}C pattern within fatty acids (Fig. 3). These data combined with steady-state calculation give a new description of metabolic steps responsible for the acetogenic lipids typical ^{13}C intramolecular distribution. In addition, the non-stochastic pattern measured in these plant fatty acids is similar to previously detected within long chain *n*-alkanes suggesting a preservation through geological time and demonstrating the interest of position-specific isotope analysis for studying the evolution of metabolic pathways.

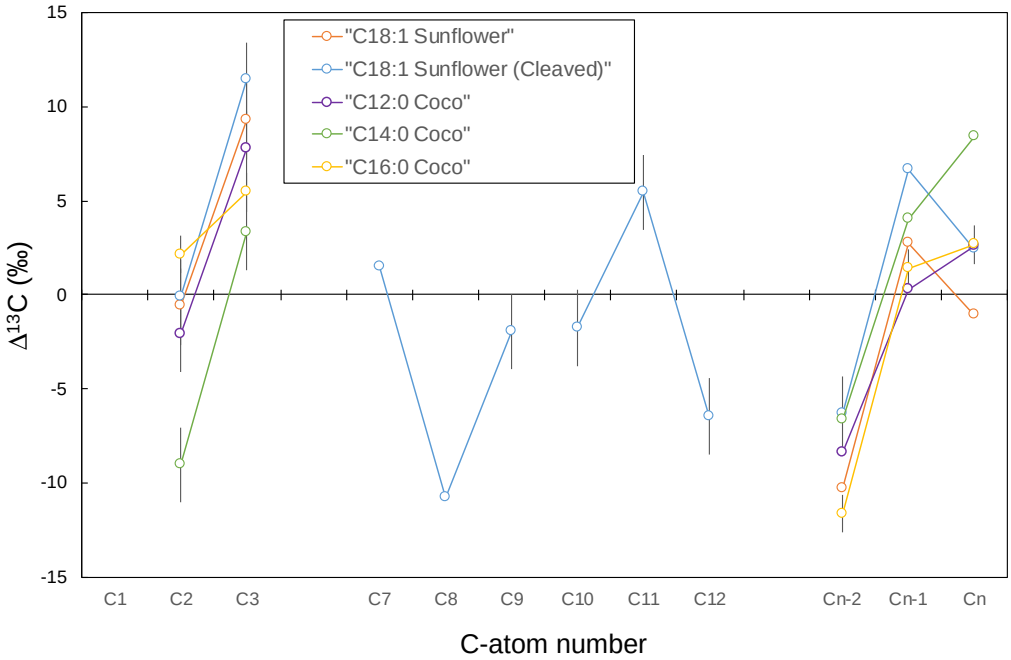

Figure 2. $\Delta^{13}C$ values of fatty acid methyl esters from coconut oil and sunflower oil. The values have been calculated omitting the carboxy position

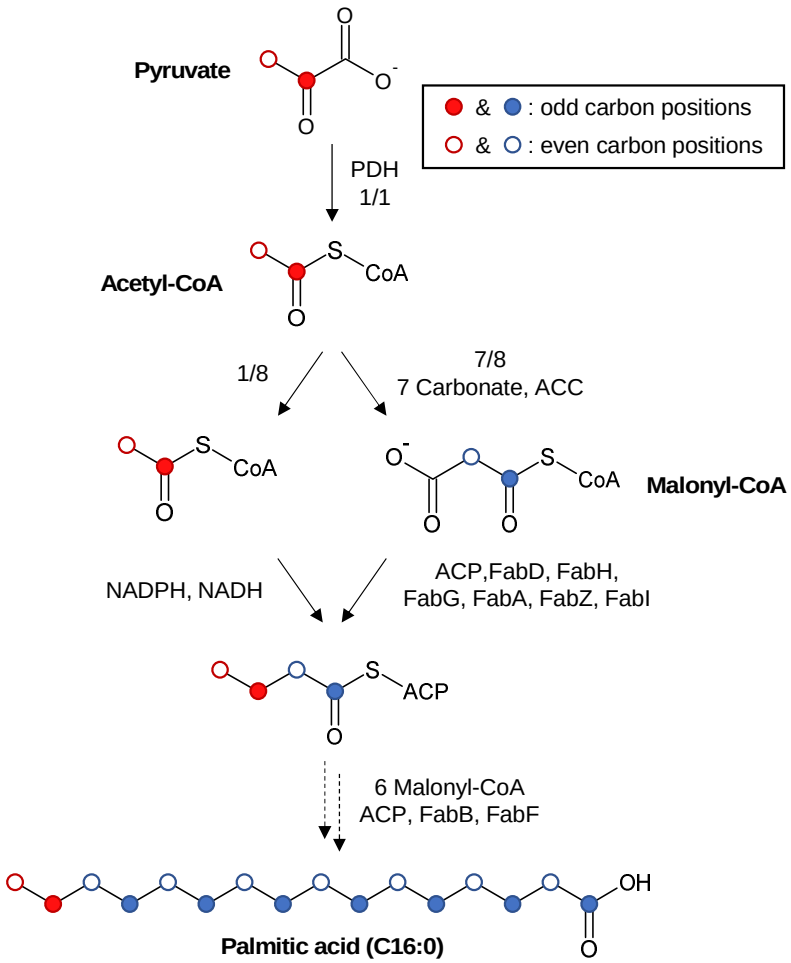

Figure 3. Summarized fatty acid biosynthesis pathway describing the origin of carbon atoms, adapted from Chan & Vogel 2010. PDH: Pyruvate dehydrogenase, ACP: Acyl carrier protein, FabD: Malonyl-CoA–ACP transacylase, FabH: b-oxoacyl synthase III, FabG: b-oxoacyl reductase, FabA: b-hydroxydecanoyl dehydratase , FabZ: b-hydroxyacyl dehydratase, FabI: enoyl reductase, FabB: b-oxoacyl synthase I, FabF: b-oxoacyl synthase II.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 M. Julien, Y. Zhao, A. Gilbert	４．巻 in prep
２．論文標題 Intramolecular isotope patterns of fatty acids measured by isotopic 13C NMR	５．発行年 2020年
３．雑誌名 In preparation	６．最初と最後の頁 0
掲載論文のDOI（デジタルオブジェクト識別子） なし	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 A. Gilbert, B. Sherwood Lollar, F. Musat, T. Giunta, S. Chen, Y. Kajimoto, C. J. Boreham, N. Yoshida, Y. Ueno	４．巻 116
２．論文標題 Intramolecular isotopic evidence for bacterial oxidation of propane in subsurface natural gas reservoirs	５．発行年 2019年
３．雑誌名 PNAS	６．最初と最後の頁 6653-6658
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.1817784116	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 T. Giunta, E. D Young, O. Warr, I. Kohl, J. L. Ash, A. Martini, S. O. C. Mundle, D. Rumble, I. Perez-Rodriguez, M. Wasley, D. E. LaRowe, A. Gilbert, B. Sherwood Lollar	４．巻 245
２．論文標題 Methane sources and sinks in continental sedimentary systems: New insights from paired clumped isotopologues 13CH3D and 12CH2D2	５．発行年 2019年
３．雑誌名 Geochim. Cosmochim. Acta	６．最初と最後の頁 327-351
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.gca.2018.10.030	査読の有無 無
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 －

１．著者名 N. Kitadai, R. Nakamura, M. Yamamoto, K. Takai, Y. Li, A. Yamaguchi, A. Gilbert, Y. Ueno, N. Yoshida, Y. Oono.	４．巻 4
２．論文標題 Geoelectrochemical CO production: Implications for the autotrophic origin of life	５．発行年 2018年
３．雑誌名 Science Advances	６．最初と最後の頁 1-7
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/sciadv.aao7265	査読の有無 無
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 －

1．著者名 M. Julien, A. Gilbert, K. Yamada, R. J. Robins, P. Hohener, N. Yoshida, G. S. Remaud.	4．巻 176
2．論文標題 Expanded uncertainty associated with determination of isotope enrichment factors: Comparison of two-point calculation and Rayleigh-plot	5．発行年 2018年
3．雑誌名 Talanta	6．最初と最後の頁 367-373
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.talanta.2017.08.038	査読の有無 無
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 －

〔学会発表〕　計5件（うち招待講演　4件／うち国際学会　0件）

1．発表者名 Alexis Gilbert
2．発表標題 Isotopologues of organic molecules: concepts, measurements and applications
3．学会等名 The 73rd Workshop of Ion-Molecule Reaction Interest Group
4．発表年 2019年

1．発表者名 Alexis Gilbert
2．発表標題 Advances in position-specific isotope analysis
3．学会等名 NASA All hands meeting April 2019（招待講演）
4．発表年 2019年

1．発表者名 Alexis Gilbert
2．発表標題 Position-specific isotope composition of natural gas hydrocarbons: recent insights and future prospects
3．学会等名 Goldschmidt 2018, Aug 12-17 2018, Boston USA（招待講演）
4．発表年 2019年

１．発表者名 Alexis Gilbert
２．発表標題 Position-specific isotope analysis of biomolecules by isotopic 13C Nuclear Magnetic Resonance
３．学会等名 Stable Isotopes in the Biosphere workshop 2019, Jan 20-25 2019, Xi ' an China（招待講演）
４．発表年 2019年

１．発表者名 Alexis Gilbert
２．発表標題 Position-specific isotope analysis of natural gas hydrocarbons
３．学会等名 Stable Isotopes in the Biosphere workshop 2019, Jan 20-25 2019, Xi ' an China（招待講演）
４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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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