The $\delta^{15}N$ of bacterial membrane lipids

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Introduction

Growing awareness that the **microbial loop** plays an important role in organic matter cycling has prompted a need for new analytical tools to study microbial interactions at a molecular level. **Compound-specific stable isotope analysis** of **amino acids** is one such method which has shown potential for the determination of microbial reworking of organic matter [e.g. 1,2,3], however, amino acids are ubiquitous and non-specific, making them less suitable for studying the microbial food web.

Nitrogen-containing membrane lipids, on the other hand, can be highly specific: Phosphatidylethanolamine (PE) for example is common in bacteria, and much less prevalent in animals. Here we have developed a method for the determination of $\delta^{15}N$ of nitrogen-containing lipids and applied it to bacterial cultures and a microbial mat.

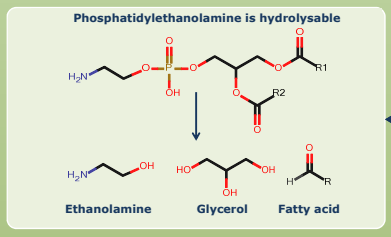
Method

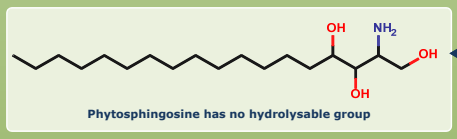
Lipids were:

- Extracted using a modified Bligh & Dyer extraction [4,5]
- Acid hydrolyzed
- Prepared for GC-analysis by derivatization of polar groups using pivaloyl chloride (modified from the CSIA method for amino acid nitrogen [6, 7])
- $\delta^{15}N$ of amino acids was determined following Chikaraishi et al. [7]

Results:

- Pivaloyl chloride reacts readily with 1° and 2° amines and hydroxyl groups





The method was tested on several different N-containing lipid standards:

Lipid	$\Delta\delta^{15}N$ (‰) (CSIA-EA)
Hydrolysable headgroups	
Phosphatidylethanolamine (PE)	-1.2 ± 0.8
Phosphatidylserine	4.9 ± 1.5 *
n-palmitoylglycine	1.4 ± 0.3
D-erythro-sphingosine-PE#	2.7 ± 0.7
Cytidine phospholipid (CDP)#	5.1 ± 0.6 *
No hydrolysable groups	
D-erythro-sphingosine	-0.4 ± 0.8
Phytosphingosine	0.3 ± 1.3
C-4-sphingosine	0.8 ± 0.9

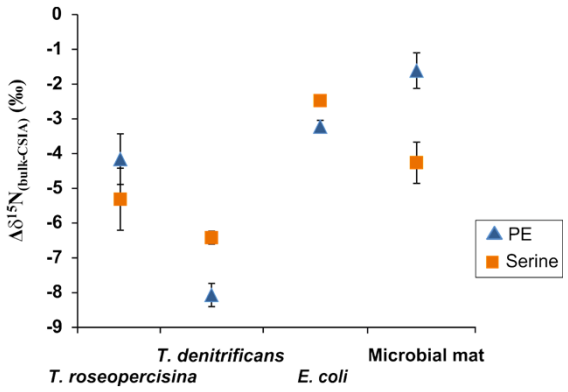
Multiple products
* Statistically significant difference (α=0.05)

- Lipids that were not hydrolyzed had small differences in $\delta^{15}N$ before and after sample workup ($\Delta\delta^{15}N$)
- Lipids that were hydrolyzed can have $\Delta\delta^{15}N$ up to 5 ‰

Conclusion: Fractionation during hydrolysis can potentially be significant

Cultures and microbial mat

The method was applied to three bacterial cultures and a microbial mat



$\delta^{15}N$ of PE and serine normalized to $\delta^{15}N$ of bulk biomass

Results:

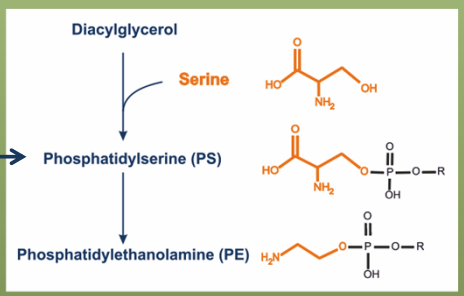
- PE is depleted in ^{15}N compared to bulk biomass by up to 8‰.
- Serine is *the* nitrogen source to PE
- The difference in $\delta^{15}N$ between PE and serine is ~2‰:
  - PE in the microbial mat is mainly influenced by heterotrophic activity
  - The $\delta^{15}N$ of serine in the microbial mat has multiple sources.
  - Equilibrium fractionation during hydrolysis

Bacterial cultures:

- *Thiocapsa roseopersicina*: purple sulfur bacteria, photoautotroph
- *Thiobacillus denitrificans:* purple sulfur bacteria, chemoautotroph
- *Escherichia coli:* Heterotroph

Microbial mat from Schiermonnikoog, Netherlands

Biosynthetic pathway of PE



As nitrogen is not involved in the reaction, biosynthetic fractionation is not expected.

Conclusions:

- PE records the $\delta^{15}N$ signal of serine from bacteria
- Compound-specific $\delta^{15}N$ determination in lipids in combination with amino acids is a new tool to trace nitrogen through biosynthetic processes.

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Questions?
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or find me in the crowds ;)

References

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