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不同氮源对海洋微藻氮同位素分馏作用的影响*

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提要 以海链藻(*Thalassiosira pseudonana*)为研究对象,分别考察了以硝酸盐、铵盐和尿素为氮源的氮同位素分馏作用。在建立相应理论模型基础上,分别计算出各个实验体系的ε值。结果表明,在藻类生长初期,$\delta^{15}N$均较低,其$\delta^{15}N$的积累主要发生在指数增长期,在稳定期达到最高,与氮源的初始$\delta^{15}N$相同;不同氮源的氮同位素分馏作用也不相同,其中以铵盐最强,硝酸盐次之,尿素最弱。考虑到实际情况下氮化合物并非单一存在,作者还进一步考察了上述3种氮源混合后对其同位素分馏作用的影响,发现混合氮源体系的表观ε值介于单一氮源时的最大与最小ε值之间,该结果较好地解释了Montoya等(1991)在Chesapeak湾的现场实验结果。

关键词 氮同位素分馏作用,混合氮源,海洋微藻
中图分类号 P731

海洋微藻的氮同位素分馏作用(Nitrogen isotope fractionation)是海洋中氮的生物地球化学循环最重要的过程之一。研究表明在硝酸盐、铵盐丰富的海洋环境中,海洋微藻的氮同位素分馏作用控制着表层海水颗粒物氮的$\delta^{15}N$(Wada *et al*, 1976; Altabet *et al*, 1991)。另外,还发现通过沉积物中^{15}N与^{14}N之比可以推测过去年代营养盐的利用情况和生产力状况(François *et al*, 1992; Altabet *et al*, 1994)。最近有学者提出,利用氮的稳定同位素比还有助于较早的判断水域富营养化以及造成富营养化的原因(McClelland *et al*, 1997),等等。尽管这些研究和发现引起了人们的重视,而对海洋中氮同位素的分馏作用研究越来越感兴趣,但迄今对有关海洋微藻对氮分馏作用的控制机理却了解的较少(Handley *et al*, 1992),并且目前对海洋微生物的氮同位素分馏作用研究大都针对某一单一氮源(以硝酸盐为主)进行的,而对于海洋中其它主要氮源(如铵盐、亚硝酸盐、有机氮等)研究的较少,更缺少接近真实情况的混合氮源的研究。为此,作者以海洋主要硅藻——海链藻(*Thalassiosira pseudonana*)为实验生物,在分别研究了不同氮源(硝酸盐、铵盐、亚硝酸盐、尿素等)对其氮同位素分馏作用影响的基础上,进一步考察了混合氮源的影响,为深入研究海洋微藻的氮同位素分馏作用机制提出了进一步的思考。

1 实验

1.1 藻种来源及其培养

海链藻藻种取自于大不列颠哥伦比亚大学(University of British Columbia)藻种培养中心,为避免天然海水中其它氮化合物对实验的干扰,本实验在Harrison等改进的人工海水中进行(Harrison *et al*, 1980; Price *et al*, 1987b)。为使藻细胞对氮化合物的充分吸收,本实验中设计为氮限制体系,培养液中的N/P和Si/N分别为4:1和2:1,藻种在连续光照(120μmol photons $m^{-2}\cdot s^{-1}$)、(18±0.5)℃温度下培养,定时取样监测活体荧光、细胞密度、颗粒氮(PN)、$\delta^{15}N_p$和营养盐等。

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1.2 颗粒氮和营养盐分析

将一定体积的样品通过450℃灼烧过的GF/F滤膜,滤膜样品分别用来检测PN和$\delta^{15}N_p$,滤液用来检测氮营养盐。其中,PN利用Fisons碳氢氮自动分析检测仪(NA1500型)测定,精确度为1%—2%;铵盐、硝酸盐和尿素的分析测定分别根据Slawyk等(1972)、Jones(1984)和Price等(1987a)等提出的方法进行。

1.3 氮同位素分析

以纯净氮气(NBS-14)为标准,利用VG PRISM质谱分析仪测定样品中的氮同位素丰度,结果以$\delta^{15}N$的形式表示:

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{std}} - 1\right) \times 1000$$

R_{sample}、R_{std}分别是样品和标准的$^{15}N/^{14}N$,测量精确度为0.17‰。本实验中所采用的氮化合物NH_4Cl、$NaNO_3$和$CO(NH_2)_2$的$\delta^{15}N$分别为-0.34‰、3.82‰和0.06‰。

2 理论模型

假设微藻对氮的吸收是不可逆的一级反应,其对^{14}N和^{15}N的反应速率常数分别为^{14}k和^{15}k。定义$^{15}k/^{14}k$为α,可表示为:

$$\alpha = \frac{^{15}k}{^{14}k} = \frac{d^{15}N_s}{^{15}N_s} \Big/ \frac{d^{14}N_s}{^{14}N_s} \qquad (1)$$

其中下标s表示溶解态N的浓度。对(1)式两边积分,设f为溶液中未吸收的溶解态N占总溶解态N的分数,由于天然丰度的^{15}N很低,所以$f \approx {}^{14}N_s/{}^{14}N_{s,0}$,下标0表示为溶解态N的初始浓度。则(1)式变为:

$$\alpha \ln f = \ln \frac{^{15}N_s}{^{15}N_{s,0}} \qquad (2)$$

根据同位素化学对同位素比率R、$\delta^{15}N$和$\delta^{15}N$‰的定义:$R = {}^{15}N/{}^{14}N \approx {}^{15}N/({}^{14}N + {}^{15}N)$,$\delta^{15}N = (R_{sample} - R_{stantard})/R_{stantard}$,$\delta^{15}N‰ = \delta^{15}N \times 1000$,式(2)可转化为:

$$\varepsilon = 10^3(\alpha - 1) = \frac{\delta^{15}N_s - \delta^{15}N_{s,0}}{\ln f} \qquad (3)$$

这里ε为单位体积(ml)^{15}N的富集系数,通常用来表示同位素分馏作用的强弱。根据^{15}N质量平衡:

$$f\delta^{15}N_s + (1-f)\delta^{15}N_p = \delta^{15}N_{s,0} \qquad (4)$$

其中下标p表示微藻吸收的^{15}N,求出$\delta^{15}N_s$并带入式(3),化简得:

$$\delta^{15}N_p = \delta^{15}N_{s,0} - \varepsilon \frac{f\ln f}{1-f} \qquad (5)$$

微藻在指数增长期对营养盐的吸收可近似为不可逆的一级反应,所以根据(5)式,通过该时期的$\delta^{15}N_p$对$f\ln f/(1-f)$做图,由斜率可求得微藻对氮同位素的分馏作用系数ε。

3 结果与讨论

3.1 不同单一氮源对海洋微藻氮同位素作用的影响

由图1a,b,c表明,海链藻在硝酸盐和铵盐中的生长速率相近,最大生长速率均为1.6d^{-1};在尿素中的生长速率较慢,最大生长速率为1.03d^{-1}。与之相对应,随着海链藻的生长,氮源逐渐降低,在藻类生长达到稳定期时消耗至零,溶解氮与颗粒氮在整个过程中保持较好的质量平衡。

以上3种氮源下的颗粒态$\delta^{15}N_p$变化规律相近,在藻类生长初期$\delta^{15}N_p$均较低,随着溶解态氮的消耗,$\delta^{15}N_p$逐渐升高,当溶解态氮消耗贻尽时,以上3种氮源体系的$\delta^{15}N_p$分别为4‰、-0.3‰、0.1‰,均接近或达到各自氮源的$\delta^{15}N_p$值(3.8‰、-0.3‰、0.1‰),表现出良好的同位素质量平衡。同位素的分馏作用主要是由于不同原子质量反应速率的差别,通常原子质量轻的反应速率高于原子质量重的(即$^{14}k > {}^{15}k$)。所以,在氮源充足的时候,藻类首先吸收的是^{14}N,随着氮源中^{14}N的减少、^{15}N比例的增加,在藻类生长后期、氮源即将消耗殆尽时,藻类则以吸收^{15}N为主,而导致细胞内^{15}N的增加,最后达到氮源原有的水平。作者在以上实验中均观察到$\delta^{15}N_p$值由低向高的变化趋势。与硝酸盐和铵盐略有不同,尿素体系的$\delta^{15}N_p$曲线有一先降后升的变化趋势,这可能是由于海链藻对尿素的氮同位素分馏作用较其它两种氮源小,而由接种带来的$\delta^{15}N_p$较显著所致。

根据公式(5),分别对上述3种氮源体系$\delta^{15}N_p$与$f\ln f/(1-f)$做图(图略),求得海链藻对上述3种氮源化合物的同位素分馏作用于表1。

表1 海链藻对硝酸盐、铵盐和尿素的同位素分馏作用

Tab.1 Nitrogen Isotopic Fractionation of nitrate, ammonium and urea on *Thalassiosira pseudonana*

富集系数	单一氮源体系			混合氮源体系	
	铵盐	硝酸盐	尿素	铵盐	硝酸盐/尿素
ε(‰)	20	4.1	0.45	10	0.70

上述结果表明,海洋微藻对不同氮化合物的同位素分馏作用差异很大,其中铵盐最显著,硝酸盐次之,尿素最弱。如前所述,目前有关氮同位素分馏作用研究多集中于硝酸盐体系,由于该作用与温度、光强、溶质浓度等环境因素有关,所报道的 ε 值并不相同,其中有关大洋体系的 ε 值多在 4.0—9.0 之间,平均估算约为 6‰(Wu *et al*,1997; Sigman *et al*,1996; Altabet *et al*,1991; Goering *et al*,1990),与本文中的结果相近。不同藻种的 ε 值也不相同,通常硅藻的 ε 值高于甲藻(Monyoya *et al*,1995)。但作者研究的海链藻 ε 值与骨条藻(*T. Weissflogii* 12‰、*S. Costatum* 9‰)相比,后者较低(Monyoya *et al*,1995; Pennock *et al*,1996),这可能是由于不同藻种氮元素通过其细胞膜的速率也不相同的缘故,如有研究表明,在相同条件下硝酸盐在骨条藻细胞内的富集量高于海链藻(Dortch *et al*,1984)。另外,海链藻在铵盐体系中的氮同位素分馏作用最强,与 Pennock 等(1996)对骨条藻的研究结果相似。作者认为这与铵盐化合物的特殊性质有关。已知 NH_4^+ 与 NH_3 之间存在酸碱平衡,而 pH 是其重要影响因素,由于藻类生长时伴随着 pH 的上升,通常由指数生长期到稳定期的 ΔpH 可达 1—2,由此可导致 NH_3 浓度升高 10 倍以上。由于 NH_3 分子较 NH_4^+ 更易穿过细胞类脂膜,从而导致铵盐体系的氮同位素分馏作用更为显著。铵盐体系这种同位素分馏作用的特性已在其它植物种类中得到了验证(Yoneyama *et al*,1993; Kleiner,1981)。与其它两种氮源相比,尿素的同位素分馏作用极小,其原因目前尚不十分清楚,可能与藻类分泌 DON 有关[1)]。

3.2 海洋微藻在混合氮源下的氮同位素分馏作用

图 1d 为海链藻在硝酸盐、铵盐和尿素同时存

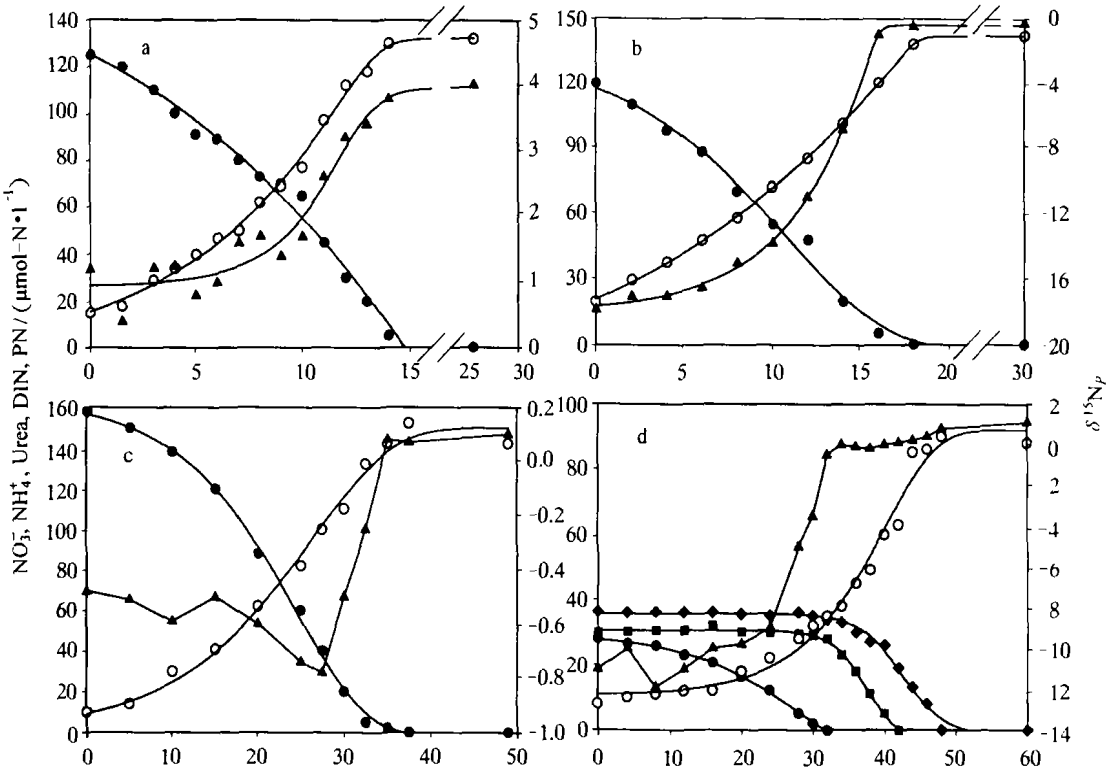

图 1 硝酸盐单一氮源体系中海链藻(*Thalassiosira pseudonana*)对硝酸盐(●)的吸收、颗粒氮(○)的增长以及与 $\delta^{15}N_p$(▲)的关系(a);铵盐单一氮源体系中海链藻(*Thalassiosira pseudonana*)对铵盐(●)的吸收、颗粒氮(○)的增长以及与 $\delta^{15}N_p$(▲)的关系(b);尿素单一氮源体系中海链藻(*Thalassiosira pseudonana*)对尿素(●)的吸收、颗粒氮(○)的增长以及与 $\delta^{15}N_p$‰(▲)的关系(c);硝酸盐(□)、铵盐(●)、尿素(■)混合氮源体系中海链藻(*Thalassiosira pseudonana*)对氮营养盐的吸收、颗粒氮(○)的增长以及与 $\delta^{15}N_p$(▲)的关系(d)

Fig. 1 Relationships between NO_3^- (●), PN(○) and $\delta^{15}N_p$(▲) in the nitrogen pool of nitrate (a); relationships between NH_4^+ (●), PN(○) and $\delta^{15}N_p$(▲) in the nitrogen pool of ammonium (b); relationships between urea(●), PN(○) and $\delta^{15}N_p$(▲) in the nitrogen pool of urea (c); relationships between nutrients, PN(○) and $\delta^{15}N_p$(▲) in the mixed nitrogen pools of nitrate(□), ammonium(●) and urea(■)(d)

1) Waser N A D, Yu Z, Harrison P J *et al*. Nitrogen stable isotope composition of dissolved organic nitrogen released by the marine diatom *Thalassiosira pseudonana*

在下藻细胞生长、氮化合物吸收和颗粒$\delta^{15}N_p$的变化曲线。结果表明,在3种氮化合物同时存在下,藻细胞首先以吸收铵盐化合物为主,待铵盐化合物消耗至零,藻细胞开始快速吸收尿素和硝酸盐,氮元素在整个营养盐消耗和细胞增长过程中保持较好的质量平衡。颗粒态$\delta^{15}N_p$的变化趋势与单一氮源时相似,但主要变化发生在藻类对铵盐的吸收阶段,$\delta^{15}N_p$从开始时的-10‰上升至铵盐化合物消耗至零时的-0.5‰,$\Delta\delta^{15}N_p$达9.5‰;在吸收尿素和硝酸盐阶段,尽管颗粒氮从32μm增长到90μm,约吸收铵盐化合物阶段的2倍,但$\delta^{15}N_p$仅从-0.5‰变化至0.8‰,$\Delta\delta^{15}N_p$约为1.3‰。根据该结果,将海链藻在上述3种氮化合物存在下的氮同位素分馏作用分为以铵盐化合物为主和以尿素/硝酸盐为主的两个阶段,分别根据公式(5)求出这两阶段的表观氮同位素分馏作用系数ε列于表1。

比较单一氮源体系和混合氮源体系的ε值可以发现,混合氮源中的铵盐表观ε值比单一氮源时铵盐的ε值低;而在尿素/硝酸盐同时吸收时的表观ε值则介于两种氮化合物分别为单一氮源时的ε值之间。作者认为这主要是由于混合氮源体系中表观ε来自于所有氮化合物的贡献:在第1阶段中,尽管藻细胞以吸收铵盐为主,但不能排除同时对硝酸盐和尿素的吸收,特别是在铵盐浓度降至很低的时候,同时吸收其它氮化合物比例就显得越来越大。由于硝酸盐和尿素的ε值远远低于铵盐的ε值,所以导致混合体系的表观ε值低于铵盐单独存在时的ε值。同理,在藻细胞同时吸收尿素和硝酸盐的第2阶段,由于硝酸盐的ε值高于尿素的ε值,导致两者存在时的表观ε值高于尿素单独存在时的ε值;反之,由于尿素的ε值低于硝酸盐的ε值,因此两者同时存在时的ε值低于硝酸盐单独存在时的ε值。这种关系可以简单用公式(6)表示:

$$\varepsilon = \sum_i a_i\varepsilon_i \qquad (6)$$

其中ε_i为氮化合物i单独存在时的ε值,a_i为其在总贡献中所占比例。由于$\sum a_i = 1$,所以$a_i \leqslant 1$,这也证明混合体系的表观ε值应介于单一体系时的最大ε与最小ε值之间,体系中的ε_i值越高,对表观ε贡献就越大,如本实验中铵盐导致颗粒物$\delta^{15}N_p$的变化远远高于其它两种氮化合物。

上述论点也较好地解释了Montoya等(1991)的现场实验结果,他们发现以铵盐为主的Chesapeak湾的ε值仅为6.5—8.0之间,远远低于实验室单独测定结果。而本实验中所得混合氮源体系的表观铵盐ε值为10.0,与之非常接近,说明其它氮化合物的存在可能是导致现场ε值较低的重要原因之一。

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EFFECT OF DIFFERENT NITROGEN POOLS ON NITROGEN ISOTOPIC FRACTIONATION DURING THE UPTAKE BY MARINE MICROALGEA

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Abstract Nitrogen isotopic fractionation during the uptake by marine microalgae is of great importance in biogeochemical cycle of nitrogen in marine system. Nitrogen isotopic fractionation controls particulate $\delta^{15}N$ in ocean surface layer where nitrate and ammonium are abundant, and the scientific interest in this issue is ever growing. However, understanding of the fractionation and its controlling mechanism are still very limited. Most studies in this field focused on single nitrogen pool, especially nitrate pool, and few of them on other major nitrogen pools, such as ammonium, nitrite and organic nitrogen. Research on mixed nitrogen pool which is close to the real case, is nearly nil. In this paper, effect of various nitrogen pools including nitrate, ammonium and urea on isotopic fractionation during the uptake by marine diatom, *Thalassiosira pseudonana*, was studied. Furthermore, effect of mixed pool of these three nitrogen compounds was determined. Finally, suggestion is made for further research on the mechanism of isotopic fractionation when nitrogen compounds were assimilated by algae. In this study, the algae were cultured in artificial seawater in order to avoid disturbance from other nitrogen compounds in natural seawater. Experimental system was designed to be nitrogen-limited, in which N/P was 4∶1 and Si/N was 2∶1, to the purpose that the nitrogen compounds were assimilated completely by algae. Particulate $\delta^{15}N$ and concentration of nitrogen compounds were measured. Based on corresponding theoretical model, the per mil enrichment factor (ε) of each experimental system was calculated. Results showed that the change pattern of $\delta^{15}N$ in particulate in three nitrogen pools was very similar. The $\delta^{15}N$ was low in the initial stage of algal growth. With consumption of dissolved nitrogen, particulate $\delta^{15}N$ increased gradually. The accumulation of the $\delta^{15}N$ occurred mainly in the period of algae exponential growth and reached the highest level during the algae steady growth period. When nitrate, ammonium and urea pools were consumed completely, their particulate $\delta^{15}N$ were 4‰, −0.3‰, 0.1‰, respectively, which were the same or very similar to the initial $\delta^{15}N$ values respectively. Isotopic mass balance was kept during the whole assimilation process. In addition, it was found that there was a great difference in magnitude of isotopic fractionation among nitrogen pools during algal uptake. ε of ammonium, nitrate and urea pools was 20‰, 4.1‰, 0.45‰ respectively, indicating that isotopic fractionation in ammonium pool was the strongest and that in urea pool was the weakest. Considering that single nitrogen pool does not exist in real circumstance, effect of isotopic fractionation in mixed pool of three compounds including nitrate, ammonium and urea was determined. The data showed that the pattern of the $\delta^{15}N$ variation in mixed pool was similar to that of single nitrogen pool. However, major change occurred when ammonium was assimilated by algae. Therefore, the process of nitrogen isotopic fractionation in mixed pool could be divided into two stages: in the first stage ammonium was basically assimilated by algae, and in the second stage nitrate and urea were assimilated. ε of ammonium and nitrate/urea pools were 10‰ and 0.7‰, indicating that compared with ε of their single nitrogen pool, apparent ε value of ammonium in mixed pool was relatively low, and apparent ε value of nitrate/urea in the mixed pool was between that of their single pools. In brief, the apparent ε of mixed nitrogen pool was between the maximum and minimum of ε of their single pools. The results suggested that apparent ε of mixed pool depended on the contribution of all nitrogen compounds, and the pool whose ε was higher could have a greater influence on the apparent ε of mixed pool. The results in this paper could explain the data of field experiment in Chesapeak Bay by Montoya *et al*.

Key words Nitrogen isotopic fractionation, Nitrogen pools, *Thalassiosira pseudonana*