

Multi-element, compound-specific isotope analysis of chloropigments: Insights into the biogeochemical cycle of the microbial mat in the shallow hypersaline environment

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In the last several decades, compound-specific isotope analysis of source-specific organic compounds (biomarkers) enabled scientists to access essential information of modern and past environments. Among numerous biomarkers, chloropigments are particularly powerful because they are synthesized only by photoautotrophs, and their tetrapyrrole nucleus is composed of carbon and nitrogen, thus providing constraints on the physiology of the photoautotrophs and the biogeochemical cycles of the photic zone from both carbon and nitrogen aspects. Another unique characteristic of the chloropigments is that they are metallo-organic complexes with Mg^{2+} as a central metal ion, potentially providing the opportunity to investigate into the biogeochemical processes that involve Mg. In this study, we investigated carbon, nitrogen, and magnesium isotopic compositions ($\delta^{13}C$, $\delta^{15}N$, $\delta^{26}Mg$) of the chloropigments isolated from the hypersaline benthic microbial mat and gypsum crusts formed in the solar salterns of Trapani, Italy. The mats and the gypsums exhibit stratification of yellow, green, and pink layers, which are inhabited mainly by photosynthetic bacteria such as cyanobacteria and purple sulfur bacteria, together with diverse chemotrophic and heterotrophic microorganisms. We measured isotopic compositions of chlorophyll *a* (Chl *a*) originating from cyanobacteria living in the upper layers, and bacteriochlorophyll *a* (BChl *a*) from purple sulfur bacteria living directly below. The relationship between Chl *a* and BChl *a* were similar for both $\delta^{13}C$ and $\delta^{15}N$ in all examined samples. Lower $\delta^{13}C$ of BChl *a* (ave. -22.9%) compared to Chl *a* (ave. -17.2%) suggests that purple sulfur bacteria are assimilating ^{13}C -depleted carbon source supplied by mineralization of the organic matter in the deeper parts of the mats and gypsums. Striking difference of $\delta^{15}N$ between Chl *a* (ave. 13.6%) and BChl *a* (ave. -2.5%) indicate that different substrate is utilized by cyanobacteria and purple sulfur bacteria. We infer that cyanobacteria are assimilating ^{15}N -enriched ammonium because $\delta^{15}N$ of nitrate ranged between -0.3 to 3.8% , indicating that nitrate is not the main nitrogen source. Unlike $\delta^{13}C$ and $\delta^{15}N$, $\delta^{26}Mg$ of Chl *a* and BChl *a* did not show any clear trend; $\delta^{26}Mg$ of Chl *a* varied between -1.77% and -0.39% and that of BChl *a* -2.13% and -0.11% , on DSM3 scale. Moreover, $\delta^{26}Mg$ of Mg^{2+} in the brine ranged between -0.88 to -1.12% , indicating that there are apparently both positive and negative isotopic fractionation between the source Mg and the chloropigments. Although more fundamental studies are necessary to understand the mechanisms determining Mg isotopic signature of the chloropigments, our results imply that $\delta^{26}Mg$ of chloropigments may vary substantially in response to the changing physiological states and environmental conditions of the photoautotrophs.

Keywords: chloropigments, carbon isotope, nitrogen isotope, magnesium isotope, microbial mat, hypersaline environment

