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博 士 学 位 论 文

南海颗粒有机物和溶解有机物宏蛋白质组学研究

Metaproteomics of particulate organic matter and dissolved organic matter in the South China Sea

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摘　要

海洋有机物（包括颗粒和溶解有机物）是全球最大的碳库之一，在全球碳循环中发挥着非常重要的作用。海洋有机物的来源、组成和降解及保护机制研究对深入了解海洋碳的生物地球化学过程和全球气候调节具有重要意义。

本论文将鸟枪蛋白质组学方法应用于海洋有机物研究领域，建立了海洋有机物宏蛋白质组学研究方法，并结合生物信息学分析，比较研究了南海不同站位和不同水层颗粒和溶解有机物的蛋白组成特征，筛选、确认了与海区、水层生物组成特征相关的蛋白质生物标志物，揭示了海区颗粒和溶解性蛋白的生源性来源、分子功能、生物学过程以及蛋白的再矿化和抗降解机制。主要研究结果如下：

（1）优化了海洋颗粒有机物蛋白提取方法，并运用鸟枪蛋白组学技术对颗粒蛋白进行了鉴定，从南海海盆区 41 m 水层颗粒有机物中鉴定到 737 个蛋白，其中 184 个为两个或两个以上肽段匹配的高可信度蛋白，如光合作用蛋白，转运蛋白，分子伴侣和孔蛋白（porins）等。除一些未知功能蛋白外，尚有相当数量的新蛋白也被检测到，这部分蛋白大约占总鉴定蛋白的 30%。海洋颗粒有机物中大量、高可信度蛋白的成功鉴定证明鸟枪蛋白组学方法可应用于海洋颗粒蛋白的研究，为海洋有机物宏蛋白质组学研究提供了理论依据和技术支撑。

（2）运用鸟枪蛋白质组学技术比较研究了南海上层（41 m 和 200 m）和中层（500 m 和 1000 m）颗粒有机物宏蛋白质组。从四个水层颗粒有机物中，一共鉴定到 3035 个一个或一个以上肽段匹配的蛋白，其中 505 个是两个或两个以上肽段匹配的高可信蛋白。蓝细菌是整个水柱颗粒蛋白的最大贡献者，而甲壳类动物和甲藻是 200 m 水层颗粒蛋白的两个主要贡献者。41 m 和 200 m 水层颗粒蛋白的亚细胞定位和生物学过程显著不同：41 m 水层含有丰富的光合作用相关蛋白，而微管蛋白和肌动蛋白则在中层积累，特别是在 200 m 水层。孔蛋白（Porin）、ATP 合成酶、营养盐转运蛋白、分子伴侣和胞外酶在颗粒有机物中被频繁检测到，但它们在整个水柱内呈现出不同的分布模式，揭示在不同水层颗粒有机物中或者在颗粒有机物下沉过程中，发生了复杂的生物地球化学过程。上层和中层颗粒蛋白的来源不同，参与细胞代谢、能量产生和转运功能的蛋白的数量和丰度随水深

迅速减少。浮游动物“粪便打包”和膜的“包裹”作用可能在颗粒蛋白的保护中起着重要作用。

（3）运用鸟枪蛋白质组学技术比较研究了来自南海表层（10 m 和 75 m）和深海（3000 m）溶解性有机物宏蛋白质组。三个溶解性有机物中，一共鉴定到 182 个蛋白，它们被 286 个专一肽段所匹配。大分子溶解有机物（LDOM, 0.2 μm -0.7 μm） 组份中的蛋白数量要显著高于小分子溶解有机物组份（SDOM, 5 kD-0.2 μm）。表层和深海 SDOM 的蛋白数量之间没有明显的差异。表层 DOM 中溶解性蛋白的来源多样，包括各种类型的细菌、浮游植物和卵菌类，而古生菌、变形菌和某些浮游植物类群是深海 DOM 的主要贡献者。参与细胞骨架组织、能量产生和转化、蛋白翻译后修饰、蛋白更新和再折叠的蛋白在表层 LDOM 丰度相当高，而参与蛋白合成相关蛋白在深海 LDOM 中更加丰富。参与物质转运和代谢、细胞壁或细胞膜或被膜的生物发生以及光合作用相关蛋白在 75 m LDOM 中相当丰富。参与氨基酸转运和代谢的 ABC 转运蛋白是 10 m SDOM 中最丰富的蛋白，而参与能量生产和转化的 methylenetetrahydromethanopterin 还原酶是 75 m 和 3000 m SDOM 中丰度最高的蛋白。在海洋垂直剖面上，溶解性蛋白的来源多样化并呈现动态的变化，每个水层都具有自己独特的蛋白，仅有非常少量、来自表层的溶解性蛋白能够被保护并输送到深海。

（4）运用鸟枪蛋白质组技术结合全球采样组合蛋白数据库比较研究了南海陆架和海盆表层小分子溶解性有机物（5 kD-0.2 μm）宏蛋白质组。从四个表层溶解性有机物中鉴定到 806 个蛋白，它们被 1477 个专一肽段（unique peptides）和 3291 幅谱图所匹配。表层小分子溶解性有机物中大部分蛋白源于细菌和病毒，仅有非常少的蛋白来自浮游植物。尽管不同细菌类群在不同采样站位表现出一定的丰度差异，但细菌类群变化不大，表明一些特定的细菌类群调节着该海区表层小分子溶解有机物的蛋白组成。一些已经在颗粒有机物和大分子溶解有机物中鉴定到的转运蛋白，如尿素 ABC 转运蛋白、氨转运蛋白、谷氨酸盐 ABC 转运蛋白、TonB-依赖受体和 porin 孔蛋白也在本研究中鉴定到。一些新型的转运蛋白，如细菌铁蛋白、变形菌视紫红质、TRAP 二羧酸转运蛋白、亚精胺周质空间结合蛋白和糖 ABC 转运蛋白则首次在溶解有机物中鉴定到。四个站位之间溶解性蛋白功能类群和种类分布非常相似，提示着蛋白功能类群和种类丰度上的差异可能主要由每个站位微生物群落结构调控。鉴定的病毒蛋白主要来自三个科：肌尾噬菌

体科、短尾噬菌体科和长尾噬菌体科。肌尾噬菌体科病毒蛋白丰度要明显高于其它两个科，暗示肌尾噬菌体科病毒可能有更强的生存能力，能够在病毒群落中迅速地发展成为优势种群。大部分病毒蛋白为病毒外部的蛋白衣壳，说明病毒粒子不同组份存在不同的更新时间。

关键词：南海；颗粒有机物；溶解有机物；鸟枪蛋白组学；宏蛋白质组学；亚细胞定位；蛋白分子功能；生物学过程

Abstract

Marine organic matter, including particulate and dissolved organic matter,is one of the largest carbon pools in the world and plays a very important role in global carbon cycle. The study of sources, composition and mechanism of degradation and protection of marine organic matters will provide in-depth understanding of marine carbon biogeochemistry and global climate regulation.

This thesis developed a high resolution and high-throughput method, shotgun proteomics to identify and characterize proteins from marine particulate organic matter (POM) and dissolved organic matter (DOM). Using this approach, protein species, composition and biological origin in POM and DOM from the South China Sea were investigated. Featured proteins which reflected the characteristics of different marine area, water depths and abiotic environment were also identified. Furthermore, according to these protein features, the mechanisms resistant to biodegradation were revealed. The main results were as follows:

(1) Using the shotgun proteomic approach combined with bioinformatic analysis, a total of 737 proteins matching one or more peptides were detected in a POM sample collected from the 41 m water layer in the basin area of the northern South China Sea (SCS). Of these, 184 were identified as high-confidence proteins matching two or more peptides, including photosynthetic proteins, transporters, molecular chaperones and porins. In addition to these proteins with known functions, a significant number of novel proteins (accounting for ~ 30% of the proteins identified) were also identified. The identification of a large number of high-confidence proteins in the POM sample demonstrated that the shotgun proteomic approach is reliable and feasible for the study of particulate proteins, and will provide a powerful tool to comprehensively investigate the nature and dynamics of POM in the ocean.

(2) Using the established shotgun proteomic approach, we characterized particulate organic matter (POM) collected from both the surface (41 m and 200 m) and meso-pelagic layers (500 m and 1000 m) in the western SCS. A total of 3035 proteins matching one or more peptides were detected from four POM samples, 505 of which were identified as high-confidence proteins matching two or more peptides. Cyanobacteria was the largest contributor throughout the water column, while crustaceans and dinophytes were the two major groups contributing to the particulate proteins in the POM collected from 200 m. Subcellular locations and biological

processes of particulate proteins varied significantly between the 41-m and 200-m layers: photosynthesis-associated proteins were highly abundant in the 41-m layer while tubulins and actins accumulated in the midwaters, especially at the 200-m layer. Porins, adenosine triphosphate (ATP) synthases, nutrient transporters, molecular chaperones, and ectoenzymes were frequently detected in the POM samples and presented different distribution patterns within the water column, revealing complex biological processes at the different water layers and/or during the sinking of POM. The sources of surface and midwater particulate proteins are different, and the cellular metabolism, generation of energy and transport processes in POM were attenuated rapidly down ocean water column. Zooplankton fecal pellet packages and membrane encapsulation might play important roles in protecting particulate proteins from degradation.

(3) Metaproteomic of dissolved organic matters(DOM, <0.7 μm in size) collected from the surface (10-m and 75-m) and bathypelagic (3000-m) layers in the SCS were investigated using the shotgun proteomic approach. A total of 182 proteins matched by 286 unique peptides were identified from three DOM samples. The number of proteins in the large DOM (LDOM, 0.2-0.7 μm fraction) was significantly greater than that in the small DOM (SDOM, 5 kD-0.2 μm fraction). There was no remarkable difference in the number of proteins between the surface and bathypelagic SDOMs. The sources of dissolved proteins were diverse in surface DOM including various bacterial and phytoplankton groups as well as Oomycetes while the Archaea, Proteobacteria, and some phytoplankton groups were the major contributors to bathypelagic DOM. Proteins involved in cytoskeleton, energy production and conversion, posttranslational modification, protein turnover, and chaperones presented high abundance in surface LDOM while proteins involved in translation, ribosomal structure, and biogenesis were more abundant in bathypelagic LDOM. Proteins involved in transport and metabolism, cell wall or membrane or envelope biogenesis, and photosynthesis were abundant in the 75-m LDOM. A urea ABC transporter assigned to amino acid transport and metabolism was the most abundant protein in the 10-m SDOM while methylenetetrahydromethano- pterin reductase involved in energy production and conversion dominated the protein profiles in the 75- and 3000-m SDOMs. The dissolved proteins in the water column are diverse and dynamic, with each layer characterized by unique proteins, and only a very minor amount of proteins from the surface are protected and transferred to deep sea.