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牡蛎铁蛋白理化特性分析与亚基的克隆表达

Analysis of the Physical and Chemical Properties of Ferritin and Cloning Ferritin Subunit in Oyster (*Saccostrea cucullata*)

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中文摘要

铁蛋白是一种广泛存在于生物体中的含铁蛋白,它具有储存和释放铁的生理功能。目前,国内外许多铁蛋白研究机构已陆续报道了有关高等动物铁蛋白的理化特性和亚基克隆表达等研究工作,但在贝类研究领域中,有关铁蛋白的结构与功能研究方面的报道并不多,尤其是牡蛎。本文从牡蛎内脏团中制备高纯度的铁蛋白,采用 SDS-PAGE 技术研究牡蛎铁蛋白的亚基类型,发现牡蛎铁蛋白由单类型亚基组成,亚基分子量约为 20 kDa,并用肽质量指纹图谱技术进一步验证了所制备蛋白是牡蛎铁蛋白。透射电子显微镜观测牡蛎铁蛋白的分子结构由蛋白壳和铁核组成,其中高电子密度的铁核(黑色球状体)位于蛋白壳(白色球空腔)的中心区域。选用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF-MS)技术直接分析牡蛎铁蛋白亚基类型,进一步确认该铁蛋白确实由单类型亚基组成,分子量为 19871.042 Da,认为这一特性不同于由 H 和 L 亚基类型组成的哺乳动物铁蛋白。释放铁动力学研究表明,牡蛎铁蛋白含铁量相对较低,每分子铁蛋白仅含有 166 个铁原子,明显低于哺乳动物铁蛋白,但铁释放规律和趋势却与哺乳动物铁蛋白很相似,即在反应时间 43 min 时出现释放铁的速率拐点,并可区分为两个不同释放铁的阶段,但均符合一级动力学反应规律。

为了揭示牡蛎铁蛋白结构与功能且能适用于环境监测和生物医药,本文克隆、表达、纯化和制备了牡蛎铁蛋白亚基,并分析了该亚基的一级、二级和三级结构,探讨了铁蛋白亚基络合顺铂能力、数量和稳定性。ICP-MS 技术研究发现,每个牡蛎铁蛋白亚基可以络合 22.9 个顺铂分子,构成了铁蛋白亚基-顺铂复合物,这为后续研制铁蛋白抗肿瘤药物且适合于临床用药,并具有减少顺铂耐药性等特色新药提供重要的科学依据。此外,还发现了牡蛎在不同浓度镉盐胁迫下,其铁蛋白 mRNA 表达量与金属镉量呈线性递增趋势,并选用 Western blotting 方法进一步佐证这一实验结果,提高实验结果的可信度,可为拓展牡蛎铁蛋白 mRNA 表达量作为监控海洋金属镉污染程度及危害性的新颖核酸标志物。进一步研究还发现了,牡蛎在弧菌感染的条件下,牡蛎铁蛋白 mRNA 的表达量也明显上调,认为牡蛎提高铁蛋白表达量的作用之一是起到抗菌效益,这一核酸标志物也可用于

选育高抗菌牡蛎品种，为减少养殖风险和增加养殖产量起着重要的作用，潜在着应用价值，预期能产生较高的经济和社会效益。

关键词：牡蛎铁蛋白；亚基新功能；核酸标志物

厦门大学博硕士学位论文摘要库

Abstract

Ferritin is a kind of protein containing iron that exists in most organisms, its major functions are storing and releasing iron. Now, many research institutes in the world have reported their research work on exploring the physical and chemical properties and cloning and expression the subunits of ferritins in mammals. However, in shellfish research field, few research reports have been reported about the structure and functions of ferritin in shellfish, especially in oyster. This paper first mainly introduces the preparation of ferritin from visceral mass in oysters, then we certify that ferritin of oyster is consisted of single type of subunit by SDS-PAGE approach, and the molecular weight of which is approximate 20kDa, and the result of PMF verifies that the protein extracted is oyster ferritin. Transmission electron microscope (TEM) is applied for observation of the structure of ferritin, in the micrograph of TEM, the iron core (the black ball) with high electronic density is localated in the center of the protein shell (the cavity of the white ball) of oyster ferritin. Matrix auxiliary laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is applied to analyze the type of oyster subunits directly, and the results of analysis further confirm that oyster ferritin comprises only one type of subunit, which is different from other types of ferritin in mammals consisted by H and L subunits. The accurate molecular weight of its subunit is 19871.042Da. The result of iron release reaction indicate that the iron content of oyster ferritin is much lower than ferritins in mammals, approximately 166 molecules of iron per molecular ferritin. However, the law of oyster ferritin iron release reaction is similar with that for mammal ferritins. That is to say, the kinetics curve of iron release reaction has inflection point at 43min, the two stages before and after the reflection point fit the kinetics equation of the first-order law.

To reveal the structure and functions of oyster ferritin and its functions can be applied to some fields, such as environmental monitoring and biological medicine. In the later part of paper, we mainly clone, express and purify the subunit of oyster

ferritin, and further study its primary structure, secondary structure and tertiary structures, furthermore, we have discussed the complexation capacity, number, and stability between the subunits of oyster ferritin and CDDP. We discovered that per molecular ferritin subunit can complex with 22.9 CDDP with the help of ICP-MS approach. The combined ferritin subunit- CDDP complex can provide important scientific grounds for exploring ferritin anti-cancer medicines and their clinical application, the novel drugs can reduce drug-resistance for CDDP. In addition, we found that the mRNA expression level has increased obviously under the stress of cadmium. Furthermore, the result of western blotting indicated that cadmium can induce the expression of ferritin in oyster, therefore, the mRNA expression of oyster ferritin can be a novel nucleic acid marker for monitoring the pollution level and harmfulness of cadmium pollution in sea. to be the biomarker monitoring cadmium pollution in sea. Finally, we found that the mRNA expression level of ferritin increases obviously after being infected with vibrio in oyster, therefore, we think that one of the functions for oysters increase ferritin expression is to take part in anti-bacteria process. Therefore, the mRNA of oyster ferritin can also be nucleic acid marker for breeding highly anti-bacterial oyster varieties, which plays an important role in reducing the risk of oyster breeding and improving breeding production. Hence, the novel nucleic acid marker has potential value for application, which is expected to produce highly economic and social benefits.

Key words: oyster ferritin; the novel functions of subunit; nucleic acid marker

1.前言

1.1 铁蛋白概述

1.1.1 铁蛋白的结构

铁蛋白广泛地分布在自然界中的动植物及微生物种群机体中,是一种调控细胞内游离铁含量的蛋白质。1884年由 Schmie deber 发现一种水溶性贮存铁的蛋白,1937年被 Laufberger 命名为铁蛋白(ferritin),1965年 Richter 等第一次从恶性肿瘤细胞株中分离出铁蛋白,并发现铁蛋白存在于脾脏、肝脏、肾脏、骨髓、心脏、胰腺、肠、胎盘等各种组织及脊椎动物的血液中^[1,2,3,4,5,6]。

在 X 射线晶体衍射技术应用于铁蛋白结构解析之前,关于铁蛋白的结构信息主要是来自于电子显微镜,这主要得益于铁蛋白非常大的分子尺寸。一般铁蛋白的直径可以达到 12 nm,而蛋白空腔的内径可以达到 8 nm^[7]。采用负染技术电镜下铁蛋白呈现较为规则的圆球形,外面一圈颜色浅,呈白色;内部颜色深,呈现灰黑色。现在知道外面浅色的部分是蛋白质壳;由于电子密度低而呈现白色。深色部分为铁核,电子密度高^[8]。

随着 X 晶体衍射技术在蛋白质结构领域的应用,越来越多的蛋白质的结构已经获得了解析,并根据衍射数据进行了蛋白质的三维重构,其中包括多种铁蛋白^[9,10]。X 晶体衍射技术显示铁蛋白的结构主要由蛋白质壳、铁核和物质隧道构成,其截面图如图 1A 所示,铁蛋白三维结构如图 1B 所示,结构相对比较简单。蛋白质壳从亚基构成上来说通常是由 24 个亚基按一定的排列方式组合而成。少数铁蛋白则由 12 个亚基构成^[11]。铁蛋白的 24 个亚基以通过 4、3、2 对称的方式构建成一个紧密而有序的壳体。反向平行的亚基二聚体对形成一个菱形的十二面体结构的平面。近来 Hsieh 等还应用磁共振力显微镜获得了和电镜相似的铁蛋白结构图^[12]。

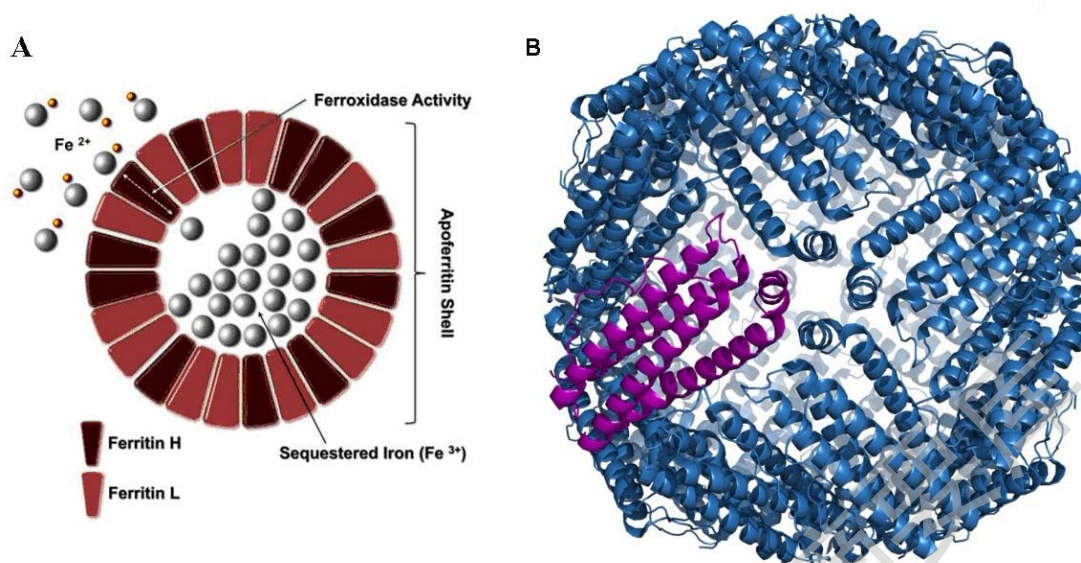


图 1 铁蛋白结构

Fig.1 The structure of ferritin

A: 模式图, B: 三维结构图

A: Mode map , B: The map of three dimensional structure

从铁蛋白亚基的种类上来看,一般认为在进化上较低等的生物含有一种类型的亚基,而进化上较高等的生物则含有两种类型的亚基,少数生物还含有三种类型的亚基,即 H、L 和 M 亚基^[13,14]。例如,细菌铁蛋白(bacterial ferritin)和鲨鱼肝铁蛋白(liver ferritin of shark, SLF) 均由单类型亚基组成^[15,16]。而哺乳动物铁蛋白蛋白壳多由 H 和 L 两种类型亚基组成^[17]。细菌铁蛋白的一大特征是其含有其他种类生物铁蛋白所没有的血红素^[18]。H 和 L 亚基的划分主要是按其生物学功能划分的。H 亚基主要负责铁的矿化和铁核晶体形成, L 亚基起着提供酸性残基, 以促进或加速铁成核的作用^[19,20]。

由于铁蛋白亚基的特殊结合方式,在蛋白壳的特定部位构成一些隧道。一般认为主要包含 3 种类型的隧道,即二相、三相隧道和四相隧道^[21]。这些隧道被认为是铁蛋白核与外界物质交换的通道^[22]。

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