文章编号：1004-0374(2011)08-0812-05

几种蓝藻光合NAD(P)H脱氢酶复合体研究的新进展

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摘　要：蓝藻 NAD(P)H 脱氢酶 (NDH-1) 是一种重要的光合膜蛋白复合体，参与 CO_2 吸收、围绕光系统 I 的循环电子传递和细胞呼吸。就几种蓝藻 NDH-1 复合体的鉴定、结构、生理功能等研究的新进展进行了综述与分析，并对今后 NDH-1 复合体的研究作了展望。

关键词：NDH-1L/L’ 复合体；NDH-1MS/MS’ 复合体；Act-NDH-1Sup 复合体；蓝藻
中图分类号：Q946; Q945.1; Q949.22　　　**文献标志码**：A

New progress in the study of several cyanobacterial NAD(P)H dehydrogenase complexes

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Abstract: Cyanobacterial NAD(P)H dehydrogenase (NDH-1) is an important photosynthetic membrane protein complex, and is essential to CO_2 uptake, cyclic electron transport around photosystem I and cellular respiration. This mini-review mainly describes and analyzes the new progress in the study of several cyanobacterial NDH-1 complexes, including their identification, structure, and physiological function. This will further help in looking ahead for the future research direction of cyanobacterial NDH-1 complexes.

Key words: NDH-1L/L’ complex; NDH-1MS/MS’ complex; Act-NDH-1Sup complex; cyanobacteria

蓝藻是一类能进行光合放氧的原核生物，也是研究光合作用的模式生物之一。一般认为，蓝藻光合膜上存在光系统 II、细胞色素 b_6f、光系统 I 和 ATP 合酶等四种光合膜蛋白复合体。1991 年，人们又在蓝藻光合膜上发现了第五种光合膜蛋白复合体，称为 NAD(P)H 脱氢酶复合体 (NDH-1)[1-2]。迄今为止，人们已经知道该复合体参与 CO_2 吸收 [3]、围绕光系统 I 的循环电子传递和细胞呼吸 [4-7]。后来的研究表明，NDH-1 复合体对蓝藻细胞的生理活动，甚至生存起着至关重要的作用 [8]。因此，该复合体是一种重要的光合膜蛋白复合体。

通过反向遗传学等的研究，发现在蓝藻细胞中至少存在两种功能截然不同的 NDH-1 复合体：一种参与围绕光系统 I 的循环电子传递和细胞呼吸；另一种则与 CO_2 吸收有关 [9-14]。2004 年以来，通过蛋白质组学等技术，在蓝藻细胞中鉴别出了多种 NDH-1 复合体。本综述就这几种蓝藻 NDH-1 复合体的鉴定、结构、生理功能等方面的新进展进行了概述与分析，并对今后蓝藻 NDH-1 复合体的研究作了展望。

1　NDH-1L/L’复合体

2004 年，通过蓝绿温和胶电泳 (Blue-native PAGE) 结合蛋白免疫印迹等技术，Herranen 等 [15] 首次在蓝藻集胞藻 6803(*Synechocystis* sp. strain PCC 6803) 光合膜中鉴别出一种相对分子质量约 4.6×10^5

收稿日期：2011-04-27；　修回日期：2011-05-04
基金项目：国家自然科学基金项目(30770175); 国家重点基础研究发展计划“973”项目(2009CB118500); 教育部重点项目(209045)
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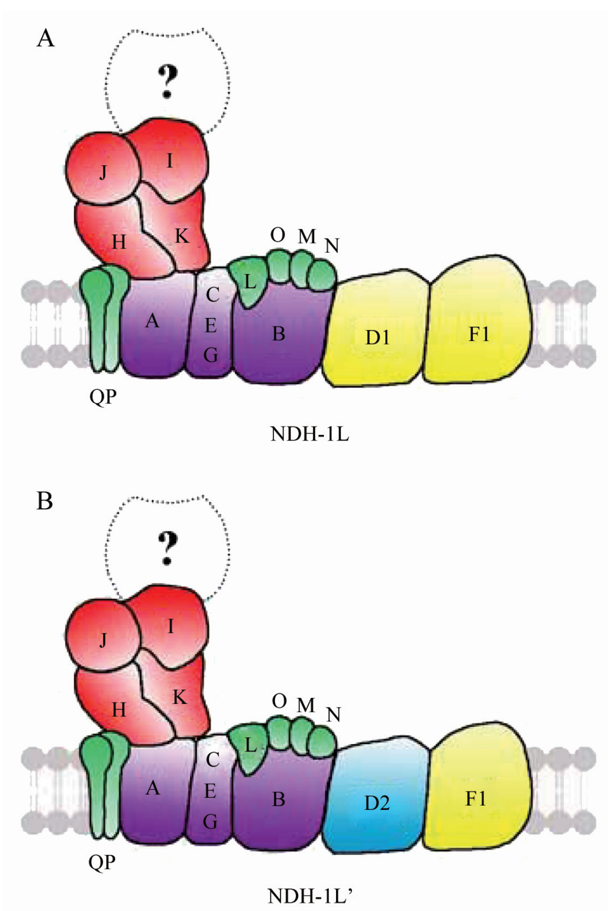
的 NDH-1L 复合体 (large NDH-1 complex) (图 1A)。随后通过蛋白质质谱分析等手段，人们在 NDH-1L 复合体中鉴定到了 15 种亚基 (NdhA ~NdhO)[15-18]。其中，NdhA~NdhG和 NdhL等 8 种亚基位于膜内，因此，它们是疏水亚基；而 NdhH~NdhK 和 NdhM ~NdhO 等 7 种亚基位于膜外，因此，它们属于亲水亚基 (图 1A)。2011 年，通过质谱等技术分析嗜热蓝藻 (*Thermosyne chococcus elongatus* BP-1)NDH-1L复合体，Nowaczyk等 [19] 鉴定到了 2 种相对分子质量稍小的新型膜亚基——NdhP 和 NdhQ。因此，迄今为止，人们发现 NDH-1L 复合体至少由 17 种亚基组成 (图 1A)。

2004 年，通过蛋白质组学结合反向遗传学的研究，Zhang 等 [20] 发现 NDH-1L 复合体参与了围绕光系统 I 的循环电子传递与细胞呼吸。2006 年，Arteni 等 [21] 用电子显微镜对通过 His6 标签纯化的 NDH-1L 复合体进行了观测，结果发现该 NDH-1 复合体呈“L”型 (图 1A)。通过反向遗传学等的预测 [10]，在蓝藻细胞中还应该存在 NDH-1L’ 复合体 (图 1B)。与 NDH-1L 复合体相比，除了 NdhD 亚基不同外，它们具有类似的亚基组成、结构以及生理功能等 (图 1A 和 1B)[22-23]。然而，可能由于蓝藻细胞中 NDH-1L’ 复合体的含量甚微或者不易从光合膜中增溶出，因此，人们至今尚未通过蛋白质组学等手段鉴定到该复合体。



A：NDH-1L复合体；**B**：NDH-1L’复合体

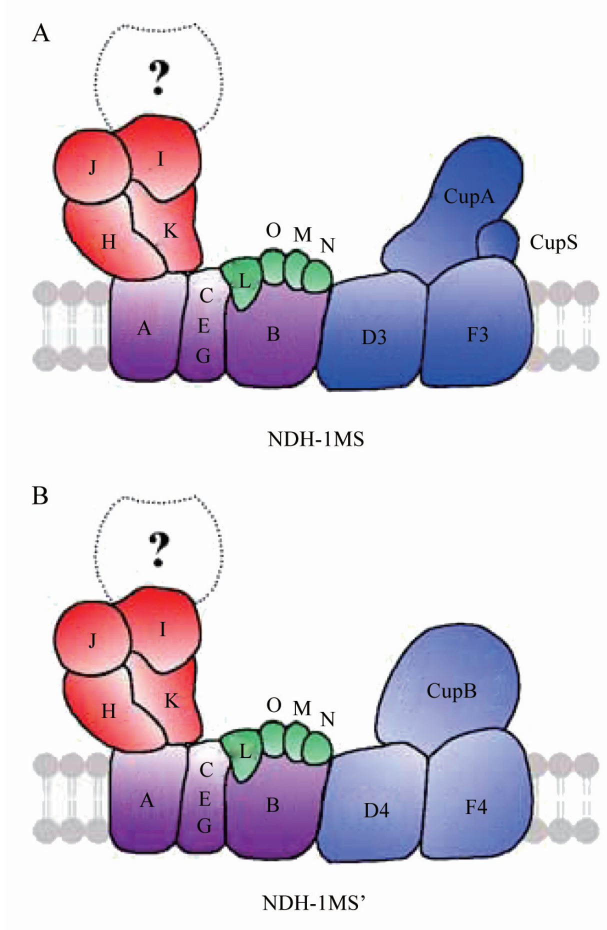
图1 蓝藻NDH-1L/L’复合体示意图

人们发现蓝藻 NDH-1L 复合体与高等植物 NDH-1 复合体不仅具有类似的结构与生理功能[23-25]，而且编码它们亚基的基因具有高度的同源性 [26-27]。因此，高等植物 NDH-1 复合体起源于蓝藻的 NDH-1L 复合体。尽管蓝藻 NDH-1L 复合体与高等植物 NDH-1 复合体均包含了 11 种 (NdhA~NdhK) 与大肠杆菌 (*E. coli*)NDH-1 复合体高度同源的亚基，但大肠杆菌中 3 种活性亚基 (NuoE、NuoF 和 NuoG)[28-30] 的同源基因在蓝藻和高等植物的基因组中缺失。因此，至今人们尚未找到蓝藻 NDH-1L 复合体中的活性亚基 (图 1 中的问号)。

2　NDH-1MS/MS’复合体

2005 年，通过蛋白纯化等技术，Zhang 等 [18] 在嗜热蓝藻光合膜上鉴定到了一种相对分子质量约为 4.9×10^5 的 NDH-1MS 复合体 (图 2A)。与 NDH-1L 复合体相比，体外的 NDH-1MS 复合体不太稳定，很容易降解为 NDH-1M 复合体 (middle NDH-1 complex, Mr = 3.5×10^5) 和 NDH-1S 复合体 (small NDH-1 complex, 约 Mr = 2.0×10^5)[18,20]。同时，利用蛋白质质谱等手段，人们在 NDH-1MS 复合体中鉴定到除 NdhD1、NdhF1、NdhP 和 NdhQ 以外的所有 NDH-1L 复合体亚基 [17-18]，并且还鉴别出了蓝藻特有的亚基 [31]：NdhD3、NdhF3、CupA 和 CupS (图 2A)。因此，不同于 NDH-1L 复合体，NDH-1MS 复合体是蓝藻细胞所特有的。

2004 年，通过蛋白质组学结合反向遗传学等的研究，Zhang 等 [18,20] 发现 NDH-1MS 复合体参与了 CO_2 吸收。然而，最近的研究结果表明 NDH-1MS 也参与围绕光系统 I 的循环电子传递 [32]。2008 年，Folea 等 [33] 用电子显微镜通过 His6 标签纯化的 NDH-1MS 复合体进行观测，结果发现该 NDH-1 复合体呈“U”型 (图 2A)。通过反向遗传学等的预测 [10]，在蓝藻细胞还应该存在 NDH-1MS’ 复合体 (图 2B)。2008 年，利用 CupB(NDH-1MS’ 复合体特有的亚基之一) 蛋白融合 His6 和 c-Myc 标签，Xu 等 [34] 成功地在集胞藻 6803 中鉴定到了一种相



A：NDH-1MS复合体； B：NDH-1MS'复合体

图2 蓝藻NDH-1MS/MS'复合体示意图

对分子质量大于 4.5 × 10^5 的 NDH-1MS' 复合体 (图 2B)。但遗憾的是，这些鉴别出的蓝藻 NDH-1 复合体均不具有氧化 NAD(P)H 的活性。

3 Act-NDH-1Sup复合体

1998 年，通过蛋白纯化等手段，Matsuo 等 [35] 在集胞藻 6803 细胞中鉴定到了一种相对分子质量为 3.76 × 10^5、具有氧化 NAD(P)H 活性的 NDH-1 亚复合体，但未能在该活性复合体中鉴别出疏水亚基，如 NdhA 和 NdhB。2003 年，邓勇等 [36] 在集胞藻 6803 细胞中分离纯化到了一种相对分子质量约为 3.0 × 10^5 的 NDH-1 复合体。该复合体不仅具有氧化 NAD(P)H 的活性，而且含有疏水亚基 NdhA。2006 年，通过 NAD(P)H- 氮蓝四唑 (NBT) 活性染色等，Ma 等 [37] 成功地在集胞藻 6803 细胞中鉴定到了一种相对分子质量约为 1 × 10^6 的 NDH-1 活性超分子复合体 (active NDH-1 supercomplex, Act-NDH-1Sup)。与上述鉴定到的几种蓝藻 NDH-1 复合体相比，Act-NDH-1Sup 具有高氧化 NAD(P)H 的活性。这一结果不仅指出了该蓝藻 NDH-1 超分子复合体具有重要的生理功能，而且包含有活性亚基，但至今尚未鉴定出。

通过反向遗传学等手段，本课题组发现 Act-NDH-1Sup 参与了围绕光系统 I 的循环电子传递和细胞呼吸 [38-39]，并且它介导的循环电子传递是减缓热胁迫条件下光系统 II 活性所必需 [40]。同时，本课题组的研究还发现了细胞内外氧化还原水平 [41] 和葡萄糖浓度的变化 [42] 调节了 Act-NDH-1Sup 的表达与活性。然而，迄今为止，人们尚不了解 Act-NDH-1Sup 复合体的其他属性，有待于进一步的研究。

4 蓝藻NDH-1复合体结构域的分析

从 NDH-1 复合体的进化角度来分析，可以把组成蓝藻 NDH-1 复合体的亚基划分为四种结构域：保守域 (conserved domain)、光合域 (oxygen photosynthetic domain)、特定域 (specific domain) 和活性域 (active domain)。保守域由大肠杆菌、蓝藻和高等植物 NDH-1 复合体中共同包含的 11 种 Ndh 亚基组成，也就是 NdhA~NdhK (图 1)[15-18,24-25,28-30]。光合域由蓝藻和高等植物 NDH-1 复合体共有的 6 种亚基构成，即 NdhL~NdhQ (图 1)[16-19,43-45]。2010 年，通过荧光蛋白标记和电子显微镜观测等手段，Birungi 等 [46] 发现 NdhL~NdhO 亚基形成了一簇 (图 1 和 2)。然而，新鉴定到的 NdhP 和 NdhQ 亚基是否也存在于这一簇中还有待于进一步研究。特定域包含了蓝藻 NDH-1 复合体特有的 7 种亚基：NdhD3、NdhD4、NdhF3、NdhF4、CupA、CupB 和 CupS (图 2)[13-14,47]。活性域则包括了那些至今尚未在蓝藻细胞中鉴定到的活性亚基 (图 1 和 2 中的问号)。

5 展望

在过去的几年中，人们在这几种蓝藻 NDH-1 复合体的鉴定、结构和生理功能等方面已经取得了许多瞩目的研究进展。然而，迄今为止，有关这几种蓝藻 NDH-1 复合体仍有许多问题亟待解决。例如，活性区到底由哪些亚基组成；如何在体外纯化出有活性的 NDH-1 复合体；如何获得高分辨率的蓝藻 NDH-1 复合体的 3D 结构，等等。这些问题的解决可能需要发展一种更为温和的蛋白质纯化技术和一种更为灵敏的蛋白质鉴定手段，而如何解决这些问

题可能就是将来蓝藻NDH-1复合体研究的努力方向。

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