Structure of intact α-carboxysome specifies role of CsoS2 in shell assembly

The carbon fixation machinery α-carboxysome of the marine cyanobacterium *Prochlorococcus* is composed of an icosahedral-like proteinaceous shell that encapsulates the enzymes RuBisCO and carbonic anhydrase. Our cryo-EM structure reveals how thousands of protein components self-assemble into the α-carboxysome and characterizes the multivalent interactions by which the scaffolding protein CsoS2 crosslinks the shell with internal RuBisCO molecules.

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The question

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyses the fixation of over 90% of the atmospheric CO_2 into biomass, but at a rather low activity. To enhance the carboxylation efficiency of RuBisCO, cyanobacteria have evolved a CO_2-concentrating mechanism that involves inorganic carbon pumps and the carboxysome[1]. Carboxysomes are well-investigated bacterial microcompartments that enclose RuBisCO and carbonic anhydrase in a semi-permeable proteinaceous shell[2]. Assembly of intact α-carboxysomes requires the ordered organization of thousands of protein components, and the scaffolding protein CsoS2 has a central role in this process. Although the structures and functions of most components have been well investigated, the assembly mechanism of intact α-carboxysomes remains elusive. We therefore asked how the scaffolding protein mediates the coordinated assembly of the shell and internal enzymes.

The discovery

We purified intact α-carboxysomes from *Prochlorococcus* MED4, which harbours the simplest operon encoding α-carboxysome components. After identifying all protein components of the *Prochlorococcus* α-carboxysome by mass spectrometry, we conducted a single-particle cryogenic electron microscopy (cryo-EM) study to investigate α-carboxysome structure and assembly. In total, around 23,400 cryo-EM images were collected and around 32,000 intact α-carboxysome particles were manually picked. An overall 7.5 Å resolution map of the intact α-carboxysome enabled us to elucidate the overall arrangement of internal RuBisCO enzymes. Block-based reconstruction[3] and focused refinement helped to improve the resolution of the shell vertex to 4.2 Å, which is sufficient for building the atomic model of the shell. Based on the 4.2 Å resolution structure, we deciphered the fine interaction pattern between the shell and the scaffolding protein CsoS2.

Prochlorococcus α-carboxysome has an icosahedral-like shell of roughly 860 Å in diameter (Fig. 1a), which is composed of 480 CsoS1 hexamers at the facets and 12 CsoS4A pentamers at the vertices. The three concentric layers of internal core each have a diameter of 720 Å, 480 Å, and 240 Å, and contain RuBisCO molecules that are tightly packed in an ordered manner (Fig. 1a). The inner surface of the shell is regularly covered by 120 CsoS2 subunits that present in two forms (Fig. 1b). CsoS2 consists of an N-terminal domain with four repetitive motifs (N1–N4), a middle domain with six repeats (M1–M6) and a C-terminal region with three repeats (C1–C3). Each M repeat of CsoS2 middle region binds to three neighbouring shell hexamers at the pseudo three-fold axis. The three conserved V/L/ITG motifs of the M repeats bind to the three shell hexamers via the His75 residue of their C-terminal β-strand. The C1 and C2 repeats of the CsoS2 C-terminal region also bind to three neighbouring hexamers via their V/L/ITG motifs. Together with previous findings[4,5], we propose a concomitant 'outside-in' assembly principle of α-carboxysomes: the inner surface of the shell is reinforced by the middle and C-terminal motifs of the scaffolding protein CsoS2, whereas the free N-terminal motifs of CsoS2 cluster to recruit RuBisCOs.

The implications

Our findings reveal the fine interaction patterns between the CsoS2 scaffolding protein and the α-carboxysome shell (Fig. 1b), highlighting a central role for CsoS2, which acts as a molecular thread that crosslinks multiple shell capsomers and internal enzymes. This study provides a detailed understanding of the assembly mechanism of α-carboxysomes and can inspire the rational design and engineering of carboxysome-based nanoreactors for broad biotechnological applications, such as for improving photosynthesis and for encapsulating enzymes for biocatalysis.

The carbonic anhydrases have also been found to be encapsulated in the α-carboxysome with a rather low abundance. However, owing to their high heterogeneity, we cannot model the low-abundance carbonic anhydrases in the present low-resolution maps of α-carboxysomes. In addition, although we observed the ordered arrangements of RuBisCO molecules within α-carboxysomes, it still remains unknown how the N-terminal motifs of CsoS2 mediate the crosslinking among RuBisCO molecules.

Clarifying the fine assembly pattern of all protein components in α-carboxysomes requires further structural studies using a combination of single-particle cryo-EM and cryogenic electron tomography. Future directions will focus on the synthetic design and repurposing of α-carboxysome structures for various biotechnological applications.

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EXPERT OPINION

"This study presents a structure of an α-carboxysome and provides new insights about α-carboxysome assembly. The high resolution of the structure makes this an important piece of work. I enjoyed reading this paper". **Georg Hochberg, Max Plank Institute for Terrestrial Microbiology, Marburg, Germany.**

FIGURE

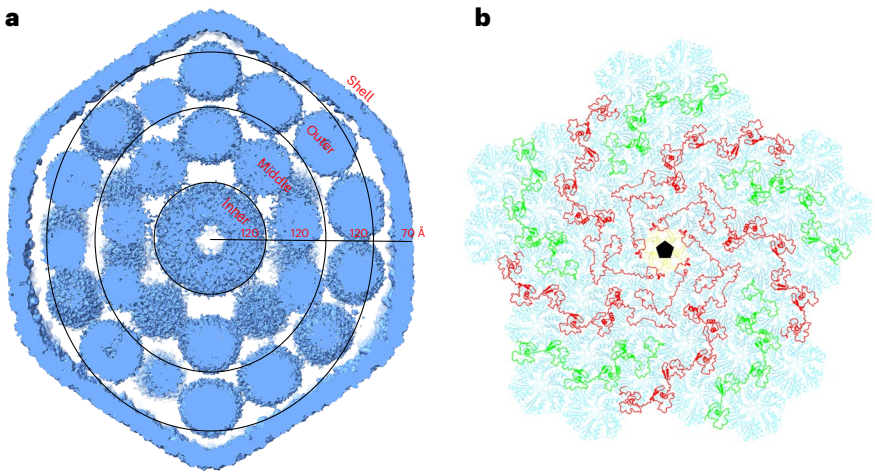

Fig. 1 | Overall architecture of *Prochlorococcus* α-carboxysome and pattern of interactions between the shell and the scaffolding protein CsoS2. a, A sliced view of the overall architecture of the four-layered structure of α-carboxysome. The distances in Å between each layer are labelled. **b**, Overall pattern of CsoS2 binding to the shell, as viewed from the inside of the shell vertex. The structures of shell CsoS1 hexamers and CsoS4A pentamers are shown in semitransparent cartoons and are coloured cyan and yellow, respectively. Ten subunits of CsoS2 are shown as cartoons, with the long and short forms coloured red and green, respectively.

BEHIND THE PAPER

Solving the cryo-EM structure of large protein complexes, especially of heterogeneous protein machineries such as the carboxysomes, has long been a great challenge for structural biologists. This project was motivated by the observation that the α-carboxysome purified from *Prochlorococcus* MED4 is the smallest α-carboxysome identified to date. This structurally simple α-carboxysome inspired us to solve the intact structure of the complex using single-particle cryo-EM. Disappointingly, the conventional cryo-EM data processing only yielded a 7.5 Å map of the intact α-carboxysome. Therefore, we proceeded with a block-based reconstruction, which can efficiently push the resolution limit for large protein machineries[3]. To our surprise, we greatly improved the resolution of the shell vertex to 4.2 Å and unexpectedly found additional densities at the inner surface of the shell that correspond to the scaffolding protein CsoS2. This progress substantially propelled the project forward and enabled us to elucidate the fine interaction pattern between the shell and the scaffolding protein of the α-carboxysome. **R.-Q.Z. & Y.-L.J.**

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FROM THE EDITOR

"The carboxysome is a carbon-concentrating structure found in cyanobacteria. This cryo-EM structure of the α-carboxysome from *Prochlorococcus*, arguably the "most abundant photosynthetic organism in the world", shows a lovely, three-layered icosahedral structure containing 108 RuBisCO molecules and associated proteins. Although cyanobacteria are not plants, ambitious attempts are being made to engineer carboxysomes into crop plants to improve their photosynthetic efficiency, making this structure extremely timely."
Chris Surridge, Chief Editor *Nature Plants*.