doi:10.1016/j.jmb.2006.09.024 J. Mol. Biol. (2006) 364, 526–535

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

# Structure of *Halothiobacillus neapolitanus* Carboxysomes by Cryo-electron Tomography

**Michael F. Schmid[1]*, Angel M. Paredes[1], Htet A. Khant[1], Ferda Soyer[2] Henry C. Aldrich*, Wah Chiu[1] and Jessup M. Shively[3]**

[1]*National Center for Macromolecular Imaging Verna and Marrs McLean Department of Biochemistry and Molecular Biology Baylor College of Medicine Houston, TX 77030, USA*

[2]*Department of Biology Izmir Institute of Technology Izmir, Turkey*

[3]*Department of Genetics and Biochemistry, Clemson University, Clemson SC 29634, USA*

**Corresponding author*

Carboxysomes are polyhedral bodies consisting of a proteinaceous shell filled with ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). They are found in the cytoplasm of all cyanobacteria and some chemoautotrophic bacteria. Previous studies of *Halothiobacillus neapolitanus* and *Nitrobacter agilis* carboxysomes suggest that the structures are either icosahedral or dodecahedral. To determine the protein shell structure more definitively, purified *H. neapolitanus* carboxysomes were re-examined by cryo-electron tomography and scanning transmission electron microscopy (STEM). Due to the limited tilt angles in the electron microscope, the tomographic reconstructions are distorted. Corrections were made in the 3D orientation searching and averaging of the computationally extracted carboxysomes to minimize the missing data effects. It was found that *H. neapolitanus* carboxysomes vary widely in size and mass as shown by cryo-electron tomography and STEM mass measurements, respectively. We have aligned and averaged carboxysomes in several size classes from the 3D tomographic reconstruction by methods that are not model-biased. The averages reveal icosahedral symmetry of the shell, but not of the density inside it, for all the size classes.



*Keywords:* carboxysomes; RuBisCO; electron cryo-microscopy; cryo-electron tomography

## Introduction

The initial source of Earth's organic carbon is atmospheric or dissolved $CO_2$, which is fixed by enzymes found in green plants, algae and autotrophic bacteria. The carbon is then spread through the food chain to all other living organisms. The most important enzyme responsible for fixing carbon is ribulose bisphosphate carboxylase/oxygenase (RuBisCO), which catalyzes the first step in carbon fixation *via* the Calvin-Benson-Bassham cycle, the covalent attachment of $CO_2$ to ribulose-1,5-bisphosphate and its subsequent cleavage into two molecules of 3-phosphoglycerate. Based on the immense biomass of photosynthetic and chemoautotrophic organisms on Earth, RuBisCO is estimated to be the most abundant protein known.

All cyanobacteria and some chemoautotrophic bacteria enhance their $CO_2$ fixation by sequestering RuBisCO into polyhedral bodies called carboxysomes.[1–4] *Halothiobacillus neapolitanus* carboxysomes are delimited by a proteinaceous shell and are filled with RuBisCO.[5–7] In previous electron microscopic studies, carboxysomes appeared hexagonal with a granular interior, a diameter of approximately 120 nm and a shell thickness of between 3 nm and 4 nm.[8,9] Although the enhancement of carbon dioxide fixation by the carboxysome has been firmly established, the exact mechanism has not yet been elucidated.

*H. neapolitanus* carboxysomes consist of ten polypeptides.[6,7] Of these, two polypeptides represent the small and large subunits of RuBisCO and six are known to make up the shell. One of these shell peptides has been identified as a unique carbonic anhydrase.[10] It is postulated that this enzyme may function to enhance carbon dioxide fixation by the carboxysome. The functions and locations of the

✠ Deceased.

Present address: A. M. Paredes, University of Texas Houston Health Science Center, Houston, TX 77030, USA.

Abbreviations used: RuBisCO, ribulose 1,5-bisphosphate carboxylase/oxygenase; STEM, scanning transmission electron microscopy; FSC, Fourier shell coefficient.

two remaining polypeptides are not yet known. Carboxysomes are rapidly formed *via de novo* mRNA and protein synthesis under low $CO_2$ conditions.[11,12] Their genes are operon-linked,[12] so it is likely the proportions of their constituents are regulated.

Although much has been published on the occurrence, physiology, biochemistry and genetics of these microcompartments/organelles,[5–7,10] only two major reports have analyzed the structure or symmetry of purified carboxysomes.[9,13] Peters concluded that the carboxysomes of *Nitrobacter agilis* obey icosahedral symmetry, based on negative stain projection images and heavy-metal shadowing of critical-point-dried carboxysomes.[13] Holthuijzen and co-workers, on the other hand, reported the shape of carboxysomes of *H. neapolitanus* to be dodecahedral.[9]

There are reports that the RuBisCO inside the shell is paracrystalline and fills the interior.[8] Others have reported that the RuBisCO molecules are arranged in one or a few layers under the surface of the shell.[9] However, these conclusions were drawn from observations of either whole bacteria or isolated carboxysome preparations that are plastic-embedded, thin-sectioned, and/or negative stained. Using Hilbert Differential Contrast electron microscopy, paracrystalline arrays were observed in intact frozen cells,[14] but it is not possible in these 2D projections to tell whether the carboxysomes are filled, or have only one or a few layers of RuBisCO under the shell. Orus *et al.*[11] reported that the state of packaging of RuBisCO inside carboxysomes can vary with environmental conditions.

It is these aspects of the carboxysome structure that we intend to clarify using cryo-electron tomography of suspensions of carboxysomes isolated from the chemoautotrophic proteobacterium *H. neapolitanus* and subsequent 3D alignment and averaging of particles computationally isolated from the tomogram. Averaging particles is necessary because of the low signal-to-noise ratio in any single instance of a particle from the tomogram, and because of the distortion introduced by the "missing wedge" of data in Fourier space. However, the search for the relative orientation of the 3D particle volumes is not a trivial step unless a 3D model of the particle with isotropic resolution already exists, as has been done.[15,16] In our study, an orientation search method that does not depend on such a prior model is used for aligning single particles from a tomogram with missing wedge data.

## Results

### Cryo-electron tomographic Tilt series images

Figure 1 shows the zero tilt image from a tilt series of a frozen-hydrated suspension of isolated carboxysomes. All the images in the tilt series are shown in Supplementary Data, Movie 1. The thin, polyhedral

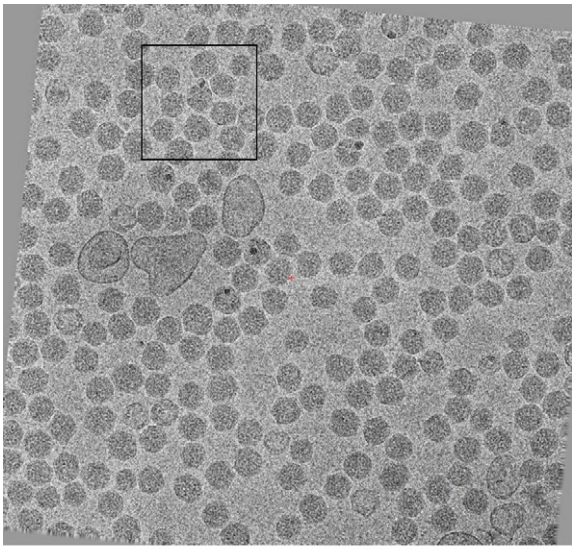


**Figure 1.** An image from the tilt series near the 0° tilt angle showing isolated carboxysomes embedded in a layer of vitreous ice. The entire series is represented in a movie as Supplementary Data, Movie 1. The box outlines the approximate area of the tomogram shown in Figure 2(a) and (b), about 400 pixels, or 432 nm on a side.

nature of the shell of the carboxysomes and their globular contents, presumed to include RuBisCO, are apparent. Also evident is that the particles appear to possess marked size variability.

### Tomographic reconstruction

Figure 2(a) is a view of a representative area of the tomogram. Four consecutive sections from the middle of the tomogram are averaged along *z*. Features annotated in this view are the shell, which is about 4 nm thick, the slightly thicker and denser vertices, the RuBisCO molecules inside the particles, and that some particles are more fully packed than others. Figure 2(b) is a stereo view of the same area of the tomographic reconstruction. The entire tomogram is shown in Supplementary Data, Movie 2. From this raw tomogram we were able to gather insight into this preparation of carboxysomes. As mentioned, we observed that a few of the carboxysome shells were completely empty. In several of them, only part of the interior was occupied. In these particles, the preferred arrangement of RuBisCO was sometimes seen as a layer or two apposed to the inside surface of the shell. However, the majority of the particles had what appears to be a homogeneous distribution of RuBisCO throughout the interior of the shell. Inspection of this entire reconstruction from a suspension of isolated carboxysomes (Supplementary Data, Movie 2) showed that there were a number of small "free" particles outside the shells that appeared very similar to the interior RuBisCO molecules of carboxysomes. Their distribution was not random. They were preferentially disposed near