Structure and function of carboxysomes

Y. A. HOLTHUIJZEN[1], J. G. KUENEN[2] and W. N. KONINGS[1]

[1] *Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands*
[2] *Laboratory of Microbiology, Delft University of Technology, Julianalaan 67A, 2628 BC Delft, The Netherlands*

Many chemolithotrophic bacteria like *Thiobacillus neapolitanus* contain polyhedral inclusion bodies (Shively et al., 1973) which contain ribulose-1, 5-bisphosphate carboxylase (RuBisCo). These microbodies are referred to as carboxysomes.

In CO_2-limited cultures of *Th. neapolitanus* the number of carboxysomes is higher than in any other culture. The degree of CO_2-limitation determines the amount of particulate RuBisCo (Beudeker et al., 1981). Beside RuBisCo, many other enzymes were reported to be present inside the carboxysomes: all the enzymes of the Calvin-cycle and a set of enzymes that made it possible for malate to act as reductant inside the microbodies (malate shuttle; Beudeker and Kuenen, 1981). These observations, however, could not be confirmed by other investigators (Cannon and Shively, 1983). The question what the role of the carboxysomes is in CO_2-fixation is therefore still not answered.

The aim of our studies is to answer this question and to analyse the structure of these microbodies.

A new method was developed for purifying the carboxysomes resulting in full separation of the carboxysomes from other cell material. In these purified carboxysomes, in addition to RuBisCo, only phosphoglycerate kinase could be detected with low activities. Stimulation of CO_2-fixation in vitro was observed after addition of ATP. The malate shuttle mechanism (Beudeker and Kuenen, 1981) could not be detected in our purified carboxysome preparations. Malate did not stimulate CO_2-fixation and no malate dehydrogenase activity was found.

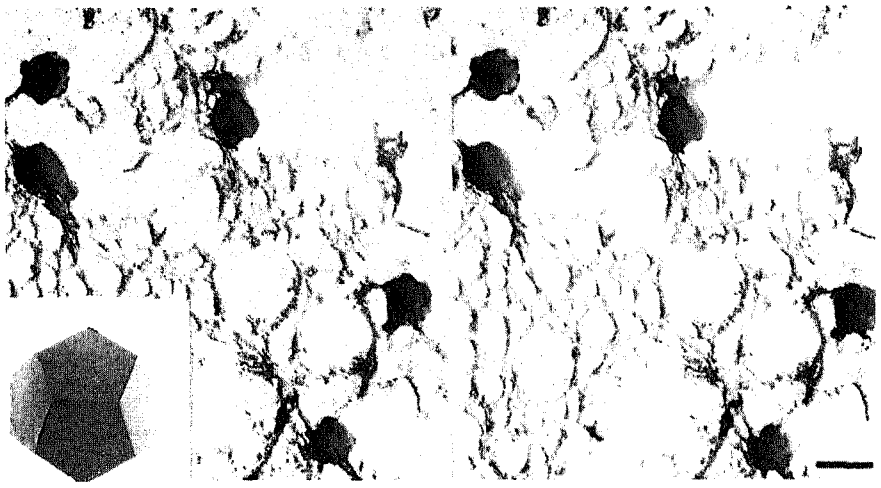

Fig. 1. Stereo-pair micrograph of carboxysomes prepared with the freeze-drying technique, with uranyl acetate (1%) as fixative. Pt-shaded, angle 26°. Bar represents 0.05 µm. Inset: model of a pentagonal dodecahedron.

The carboxysomes seem to have a pentagonal dodecahedric structure in Pt-shaded electron microscope pictures (Fig. 1). Preparations treated with 1% uranylacetate or 3% ammoniummolybdate showed an orderly arrangement of the RuBisCo molecules inside the carboxysomes. The 10 nm molecules are surrounded by a shell that maintains its shape after breakage.

The number of proteins present in the carboxysomes has not been completely elucidated. Crossed immunoelectrophoresis revealed the presence of three proteins and isoelectric focusing and SDS-PAGE indicate that eight proteins and thirteen polypeptides are present. The shell of the carboxysomes consists of four glycoproteins.

It has been reported that carboxysomes of *Nitrobacter winogradskyi* and *N. agilis* contain DNA (Westphal et al., 1979). No evidence for the presence of DNA in carboxysomes of *Th. neopolitanus* could be obtained. However, large amounts of DNA were attached to and perhaps penetrating the carboxysomes. This DNA appears to be of chromosomal origin.

BEUDEKER, R. F., CODD, G. A. and KUENEN, J. G. 1981. Quantification and intracellular distribution of ribulose-1,5-bisphosphate carboxylase in *Thiobacillus neapolitanus*, as related to possible functions of carboxysomes. — Arch. Microbiol. **129**: 361–367.

BEUDEKER, R. F. and KUENEN, J. G. 1981. Carboxysomes: 'calvinosomes'? — FEBS Lett. **131**: 269–274.

CANNON, G. C. and SHIVELY, J. M. 1983. Characterization of a homogenous preparation of carboxysomes from *Thiobacillus neapolitanus*. — Arch. Microbiol. **134**: 52–59.

SHIVELY, J. M., BALL, F., BROWN, D. H. and SAUNDERS, R. E. 1973. Functional organelles in prokaryotes: polyhedral inclusions (carboxysomes) of *Thiobacillus neapolitanus*. — Science **182**: 584–586.

WESTPHAL, K., BOCK, E., CANNON, G. and SHIVELY, J. M. 1979. Deoxyribonucleic acid in *Nitrobacter* carboxysomes. — J. Bacteriol. **140**: 285–288.