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Activity of ribulose-1,5-bisphosphate carboxylase in intact and disrupted carboxysomes of *Thiobacillus neapolitanus*

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1. SUMMARY

Carboxysomes isolated from *Thiobacillus neapolitanus* remained intact in buffers of low osmolarity during the first 30 s sonication. The ribulose-1,5-bisphosphate carboxylase activity of these (intact) carboxysomes was 2.1–2.4 μ moles CO₂ fixed/min (mg protein). In these intact carboxysomes ribulose-1,5-bisphosphate carboxylase did not interact with antibodies against the large subunit, indicating that this subunit is not exposed to the outer surfaces. Sonication of the carboxysomes for periods exceeding 30 s caused gradual disruption of the carboxysomes, a decrease of the enzyme activity and a release of ribulose-1,5-bisphosphate carboxylase. In preparations containing about 50% disrupted carboxysomes the enzyme activity was decreased by 93%. This large decrease of enzyme activity is most likely caused by a dissociation of the subunits of ribulose-1,5-bisphosphate carboxylase in the carboxysomes.

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2. INTRODUCTION

In *Thiobacillus neapolitanus* and many other autotrophic bacteria carboxysomes are found. These inclusion bodies, 120 nm in diameter, contain the first enzyme of the Calvin cycle, D-ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.139 (RuBisCO)) ([1,2], for a review see [3]). In cells of *T. neapolitanus* grown in continuous culture a maximal number of carboxysomes was found during CO₂ limitation and only a few during thiosulphate limitation [4]. Carbon dioxide uptake experiments in starved cells of *T. neapolitanus* indicated that CO₂-limited cells had a much higher potential for CO₂ fixation than thiosulphate-limited cells [5]. When CO₂-limited cells of *T. neapolitanus* were switched to thiosulphate limitation a rapid degradation of carboxysomes and a subsequent decrease of RuBisCO activity was found [4]. Artificial degradation of isolated carboxysomes by sonication resulted in release of only the large subunits of RuBisCO; the small subunit was always found together with the small proteins [1]. This shell is composed mainly of glycoproteins and determines the outer shape and the stability of the carboxysomes [1].

The present study has been undertaken to compare the catalytic activity of RuBisCO in intact and disrupted carboxysomes and to determine whether the large subunits of RuBisCO are exposed to the outer surface of carboxysomes.

3. MATERIALS AND METHODS

3.1. Organism and growth conditions

Thiobacillus neapolitanus strain X, was grown in minerals thiosulphate medium in a 1 l chemostat at pH 6.8 under CO₂ limitation at 28°C as described previously [6].

3.2. Methods

Isolation of the carboxysomes. Cells were harvested and carboxysomes were isolated as described previously [1]. The isolated carboxysomes (15 mg protein/ml) were stored at 4°C in 20% sucrose (w/w) in 10 mM Hepes/KOH (pH 8.0), 20 mM MgCl₂, 1 mM dithiothreitol and 2 mM phenylmethylsulphonyl fluoride, a serine protease inhibitor.

Isolation of the large subunit from D-ribulose-1,5-bisphosphate carboxylase (RuBisCO). Shell and contents of the carboxysomes were separated as described previously [1]. The supernatant of the centrifuged suspension of the disrupted carboxysomes contained only the large subunit of RuBisCO as indicated by sodium dodecyl sulphate polyacrylamide gel electrophoresis [1].

Isolation of antibodies. Antibodies against the isolated large subunit of RuBisCO were raised and isolated as described previously [1].

Enzyme activities. RuBisCO (EC 4.1.1.39) activity was assayed by the method of Beudeker et al. [7].

Electron microscopy. Intact or broken carboxysomes were negatively stained with 1% uranylacetate and examined with a Philips EM 201 or 300.

Protein determination. Protein was determined by the method of Lowry et al. [8] using bovine serum albumine as a standard.

Immunogold-labeling. The immunogold-labeling procedure was performed as described by Douma et al. [9] with intact and disrupted carboxysomes which were incubated with protein A-gold-labeled antibodies against the large subunit of RuBisCO.

4. RESULTS AND DISCUSSION

The ribulose-1,5-bisphosphate carboxylase (RuBisCO) activity was determined in intact carboxysomes and in partially or totally disrupted carboxysomes. In some preparations the exposure of the RuBisCO molecules to the outside was examined by immunogold-labeling.

When carboxysomes were suspended in buffers without 20% sucrose as osmotic stabilizer they remained intact for several hours. Even when the carboxysomes were sonicated for 30 s, electron-microscopically no disruption could be seen (Fig.



Fig. 1. Carboxysomes negatively stained with 1% uranyl acetate. (a) Carboxysomes, sonicated for 30 s; (b) carboxysomes sonicated 3 times 30 s; (c) carboxysomes sonicated 20 times 30 s. Bar represents 0.1 μ m.

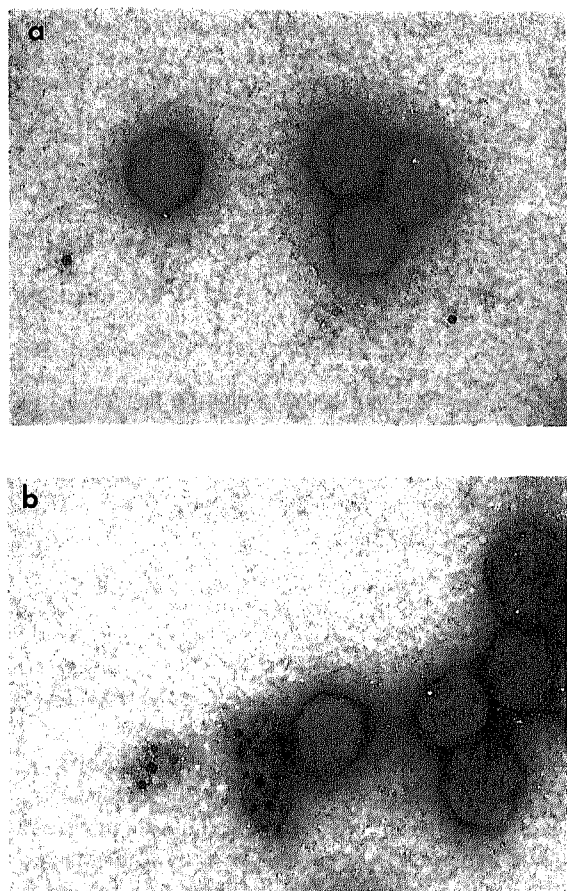


Fig. 2. Immunogold-labeling of carboxysomes with antibodies against the large subunit of ribulose-1,5-bisphosphate carboxylase. (a) Intact carboxysomes; (b) a carboxysome broken by 3 times 30 s sonication. Bar represents 0.1 μm .

1a). In agreement with previously made observations [7,10,11] these preparations retained the RuBisCO activity of intact carboxysomes which was 2.1–2.4 $\mu\text{moles CO}_2$ fixed/min (mg protein).

Protein A-gold-labeled antibodies against the large subunit of RuBisCO were used to study the accessibility of the large subunit from the outside of the carboxysomes. No gold-labeled antibodies were found to react with the intact carboxysomes, not even after 30 s sonication (Fig. 2a). These observations confirm previous electron microscopic observations which showed the RuBisCO molecules in a monolayer against the inside of the carboxysomal shell [2].

When the carboxysomes were subjected to longer sonication periods (3 times 30 s) they were partially disrupted. In approximately 50% of the carboxysomes gaps were formed in the carboxysomal shell through which particles were seen to be released (Fig. 1b). These particles could be identified as RuBisCO molecules by immunogold-labeling (Fig. 2b). The RuBisCO enzyme activity of these suspensions, containing 50% disrupted carboxysomes, was reduced by more than 92% (to 160 nmoles CO_2 fixed/min (mg protein)) in comparison to the activity of intact carboxysomes. When the sonication of the carboxysomes was continued for even longer periods (20 times 30 s) the carboxysomal structures disappeared completely (Fig. 1c), the enzyme activity dropped to 52 nmoles CO_2 fixed/min (mg protein) and many free RuBisCO molecules could be found in the preparation by immunogold-labeling (data not shown). This loss of activity is, probably, not due to denaturation since Beudeker et al. [7] showed that (cytoplasmic) RuBisCO remained enzymatically active during repeated sonication. Recently we have found that the RuBisCO molecules released upon disruption of the carboxysomes consisted solely of the large subunit [1]. The small subunit was found to be attached to the carboxysomal shell. This dissociation of the large and small subunits of RuBisCO might explain the large decrease of the enzymatic activity upon disruption of the carboxysomes. Dissociation of the subunits has been reported previously to result in loss of RuBisCO activity in several autotrophic organisms [12–14]. Possibly this dissociation is also the cause of the observed decrease in CO_2 fixation by RuBisCO in *T. neapolitanus* when the cells are switched from CO_2 limitation to CO_2 excess [5].

The reduction in RuBisCO activity after 3 times 30 s sonication was surprisingly large since only 50% of the carboxysomes were seen to be disrupted with (the large subunits of) the RuBisCO molecules coming out. Instead of a 50% decrease of the total RuBisCO activity a decrease of 92% was measured. It may be speculated that the subunits of the RuBisCO become already dissociated inside the carboxysomes upon repeated sonication.

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