Localization of carbonic anhydrase in the cyanobacterium

*Chlorogloeopsis fritschii*

(Carbonic anhydrase; *Chlorogloeopsis fritschii*; carboxysomes; cyanobacteria)

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1. SUMMARY

90% of the carbonic anhydrase (CA) activity recovered from *Chlorogloeopsis fritschii* cells, when broken under conditions which favour the isolation of carboxysomes, is particulate. Subsequent sucrose density gradient centrifugation of the carboxysome-containing pellet produced a sharp band of CA, well separated from the carboxysomes and thylakoids. The implications of these findings for the possible functions of carboxysomes and location of CA are discussed.

2. INTRODUCTION

Carboxysomes (polyhedral bodies) occur in several members of the colourless sulphur-oxidising bacteria, ammonia- and nitrite-oxidising bacteria and in all members of the cyanobacteria so far examined [1,2]. Polyhedral inclusions, which may be carboxysomes, have also been found in the thermophilic hydrogen bacterium *Pseudomonas thermophila*, in members of the chlorophyll b-containing photosynthetic prokaryote *Prochloron*, and in cyanelles (see [2]). The apparent restriction of carboxysomes to autotrophic prokaryotes, which fix CO₂ via the Calvin cycle, suggests that the function of these inclusions may be specifically related to the autotrophic mode of life. This possibility is greatly strengthened by the fact that in all cases examined, the carboxysomes contain the CO₂-fixing enzyme of the Calvin cycle, D-ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [3–7]. Between 7 and 13 polypeptides have been detected by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) of SDS-dissociated isolated carboxysomes [7–9]. Two polypeptides have been identified as the large (L) and small (S) subunits of RuBisCO [7–9] which accounts for more than 50% of total carboxysomal protein. A further two polypeptides (10 and 15 kDa) have been assigned to the membrane of *Thiobacillus neapolitanus* carboxysomes [9], but the identities of the other carboxysomal polypeptides remain unknown.

Possible functions for carboxysomes include a CO₂-concentrating mechanism to accumulate CO₂ in the vicinity of RuBisCO [2,10]. Such a mechanism would favour the carboxylase rather than the oxygenase activity of the enzyme, assuming that carboxysomal RuBisCO is catalytically active in vivo, and could be supported by carbonic anhydrase (CA), which has recently been purified.

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from the cyanobacterium *Anabaena variabilis* [11]. *A. variabilis* CA has a *M*<sub>r</sub> of 42000 ± 5000, as measured by PAGE. We have isolated carboxysomes from *C. fritschii* and although CA has not been reported from this cyanobacterium, a 43 kDa polypeptide is among the unidentified carboxysomal components [7]. In this paper we report on the presence and localization of CA in *C. fritschii*.

3. MATERIALS AND METHODS

3.1. Organism and growth conditions

*C. fritschii* 1411/16 (Culture Centre of Algae and Protozoa, Cambridge, U.K.) was grown photoautotrophically in pure batch culture in BG-11 medium including nitrate [12]. Cultures were sparged with air and growth conditions were as detailed previously [13].

3.2. Preparation of cell-free extracts and carboxysome isolation

Late exponential phase cells were used throughout. Cell-free extract preparation and carboxysome isolation, using 10–60% (w/w) sucrose density gradients, were performed using 25 mM K-phosphate buffer, pH 8.4. All other conditions and methods were as described previously [7].

3.3. Assay of carbonic anhydrase (EC 4.2.1.1)

An electrometric assay based on that described by Nelson et al. was used [14]. Assays were per-

**Table 1**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total CA activity (U)</th>
<th>Protein content (mg per fraction)</th>
<th>CA specific activity</th>
<th>Relative distribution of CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>40000×g·1 h supernatant</td>
<td>53</td>
<td>146</td>
<td>0.36</td>
<td>10.03%</td>
</tr>
<tr>
<td>40000×g·1 h pellet</td>
<td>475</td>
<td>89</td>
<td>5.35</td>
<td>89.97%</td>
</tr>
</tbody>
</table>

* U, unit of activity = (ΔpH/ΔpH<sub>0</sub> - 1)×10 where ΔpH and ΔpH<sub>0</sub> were the rates of pH change of the enzyme-catalyzed and -uncatalyzed reactions; for details see section 3.3.

b Units per mg protein.
formed in a 10 ml ice-cooled vessel. The reaction mixture contained 8 ml 25 mM K-phosphate buffer, pH 8.4 and 1 ml enzyme solution. 1 ml CO₂-saturated distilled deionized H₂O was added to start the reaction. The initial reaction rate was measured using a Kent EIL 7055 pH-meter connected to a recorder with a chart speed of 1 cm·s⁻¹. 1 pH unit corresponded to 50 cm on the paper. For the uncatalyzed reaction, 1 ml buffer was substituted for the enzyme solution. Units (U) of enzyme activity were calculated according to 

\[ U = \left( \frac{\Delta pH}{\Delta pH_0} - 1 \right) \times 10 \]

where \( \Delta pH \) and \( \Delta pH_0 \) were the rates of pH change of the enzyme-catalyzed and -uncatalyzed reactions respectively. CA activity was quantitated on a protein basis, protein being assayed as described before [7].

4. RESULTS AND DISCUSSION

CA activity was readily detectable in broken cell-free extracts of C. fritschii (Table 1), although no activity was observed in whole-cell assays containing up to 8 mg protein. Differential centrifugation of the cell-free extract was performed and CA activity found in both the 40000 × g·1 h supernatant and pellet fractions. Enzyme activity per unit protein was about 15 times higher in the pellet than soluble fraction and almost 90% of the extractable CA activity was particulate (Table 1).

It should be noted that the C. fritschii cells were harvested from late exponential phase photoautotrophic batch culture when carboxysomes are abundant, as evidenced by a high distribution of RuBisCO in the carboxysomal versus the cytoplasmic pool [7,15]. Furthermore, the cells were broken under conditions which favour carboxysome recovery [7]. Since the CA activity was almost entirely particulate, it was of interest to determine whether the enzyme was associated with the carboxysomes. Centrifugation of the gently resuspended 40000 × g pellet into linear density gradients of sucrose produced a sharp band of CA activity focussed in the 30% (w/w) sucrose region (Fig. 1a), although low activity was found throughout the gradient. The CA peak was clearly above the chlorophyll-containing thylakoid band, but below the bulk band of proteins at the top of the gradients (Fig. 1a and b). Peak gradient fractions stored at 4°C still showed 75% of initial activity after 7 days.

The sucrose gradients used here for the recovery and localization of CA were made in phosphate buffer without additions, rather than the usual Mg²⁺- and sulphydryl-containing buffer used for RuBisCO assays, since these RuBisCO requirements had been reported to suppress CA activity in A. variabilis extracts [11]. Indeed, our initial attempts to measure CA in C. fritschii extracts prepared in RuBisCO assay buffer [7] were unsuccessful (not shown). Previous studies with C. fritschii [7,16] and Anabaena cylindrica [4] have consistently localized the carboxysome band below the thylakoids after sucrose density gradient centrifugation. The focussing of particulate CA into a band above the thylakoids indicates that this enzyme is not specifically associated with C. fritschii carboxysomes.

Early work by Ingle and Colman demonstrated the presence of CA in 4 species of cyanobacteria, but only soluble CA activity was detected in the single localization study performed [17]. These workers concluded that CA is a cytoplasmic enzyme in Oscillatoria. Yagawa et al. have, however, found that 50–70% of the CA activity in extracts of two A. variabilis strains was particulate but CA activity was only detected in the supernatant fraction from a third strain of this organism [11]. The location of the particulate CA of A. variabilis was not investigated further. The possibility that T. neapolitanus carboxysomes may contain CA has been considered [9]. Although CA was intermittently detectable in crude cell-free extracts from this source, no enzyme activity was found in intact or broken carboxysomes, or in high-speed supernatants. No conclusion was therefore possible, although it is noted that T. neapolitanus carboxysomal CO₂ fixation was unaffected by the CA inhibitor, acetolamide [9].

The present studies have established that C. fritschii CA is almost entirely particulate and that the enzyme is not specifically associated with the carboxysomes. The possibility that carboxysomes have a mechanism for concentrating dissolved inorganic carbon for fixation by RuBisCO cannot be discounted. However, if carboxysomes do have
such a role then it would be unlikely to involve either a C₄-dicarboxylic acid fixation pathway [2,10], or CA. The high particulate CA in C. fritschii may be involved in the concentration of inorganic carbon, but the dehydration of bicarbonate to produce CO₂, the RuBisCO substrate [2], cannot be assumed to occur specifically in the carboxysomes. If CA was exclusively associated with these inclusions, then this would suggest that carboxysomal RuBisCO was active in CO₂ fixation in vivo and that the carboxysomes were the only, or principal, site of CO₂ fixation. However, the fact that CA is located elsewhere in the cell indicates that the carboxysomes are not the sole site of CO₂ fixation, if they are catalytically active at all.

The identity of the cell inclusions or membranes associated with C. fritschii CA is unknown. The inner or outer cytoplasmic membranes would seem to be possible sites, although until recently, information on these structures in cyanobacteria was lacking. Omata and Murata [18] have recently separated the cytoplasmic membranes from the thylakoids in Synechocystis extracts and characterization of these individual membrane classes in this cyanobacterium is now possible.

ACKNOWLEDGEMENTS

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REFERENCES