SHORT COMMUNICATION

Immunoelectronmicroscopic Localization of Calvin Cycle Enzymes in Chlorogloeopsis fritschii

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(Received 23 May 1985)

Antisera against the Calvin cycle enzymes D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and phosphoribulokinase (PRK) have been used in immunogold electronmicroscopy studies on cell sections of the cyanobacterium Chlorogloeopsis fritschii. RuBisCO antiserum consistently labelled the carboxysomes (polyhedral bodies) and the cytoplasm. PRK antiserum-labelling occurred in the cytoplasm but not in the carboxysomes. The data agree with in vitro enzyme localization studies, and confirm that both enzymes occur in the cytoplasm and that RuBisCO, but not PRK, also occurs in the carboxysomes of C. fritschii.

INTRODUCTION

We have isolated carboxysomes (polyhedral bodies) from the cyanobacterium Chlorogloeopsis fritschii and have obtained evidence in vitro that these inclusion bodies contain the CO₂-fixing enzyme of the Calvin cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Lanaras & Codd, 1981a, 1982). The association of RuBisCO with carboxysomes has also been demonstrated for other autotrophs, including colourless sulphur-, nitrite- and ammonia-oxidizing bacteria (Codd & Marsden, 1984). The possibility was raised that other Calvin cycle enzymes may also be located in carboxysomes and evidence for, and against, this concept has been obtained for Thiothrix pseudopriapulus (Beudeker & Kuenen, 1981; Beudeker et al., 1981; Cannon & Shively, 1983). Our interest in the location of the second enzyme essential for and unique to the Calvin cycle, phosphoribulokinase (PRK), was prompted by the presence in dissociated C. fritschii carboxysomes of a cryptic 39 kDal polypeptide (Lanaras & Codd, 1981a) with almost the same molecular mass as the subunits of purified C. fritschii PRK (40 kDal; Marsden & Codd, 1984).

PRK activity and enzyme protein were almost entirely in the soluble (cytoplasmic) fraction of C. fritschii cells, broken under conditions which favour carboxysome recovery, and no association of the enzyme with carboxysomes was found (Marsden et al., 1984). Nevertheless, the difficulties of carboxysome isolation make desirable a complementary approach to subcellular enzyme localization. Here, we have used immunocytochemical labelling, involving colloidal gold with RuBisCO and PRK antisera, to examine the distribution of Calvin cycle enzymes in thin sections of C. fritschii cells.

METHODS

Organism and growth. Chlorogloeopsis fritschii 1411/16, from the Culture Centre of Algae and Protozoa, Cambridge, UK, was grown as detailed before (Codd & Stewart, 1973), except that cells were harvested during early stationary phase.

Cell fixation. Cells were harvested by centrifugation at 2000 g for 10 min and fixed in phosphate buffer (0.1 M, pH 7.2) containing 2% (v/v) glutaraldehyde for 2–6 h at room temperature. Excess glutaraldehyde was removed by...
Fig. 1. Localization of RuBisCO and PRK in C. fritschii. Cell sections were labelled with 20 nm colloidal gold particles after treatment with RuBisCO antiserum (a) and PRK antiserum (b). CA, carboxysome. Bar markers represent 1 μm.

washing twice for 15 min in the same buffer. The cells were dehydrated in a graded ethanol series (50%, 2 × 15 min, followed by 15 min each of 70, 90, 95 and 100% v/v, ethanol), and then embedded in LR White Resin (London Resin, Basingstoke, Hants, UK) for 2 × 30 min, followed by an 18 h incubation. Samples were then polymerized at 60 °C for 20 h in glycerine moulds.

Cell sectioning and labelling. A modification of the method of Beesley et al. (1984) was used. Cells were sectioned onto uncoated gold grids (400 mesh) using a diamond knife. The grids were incubated on Tris/HCl buffer (50 mM, pH 7.4) containing 1% (w/v) bovine serum albumin, 1% (v/v) Tween-20 and 0.2% (w/v) gelatin, before incubation
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Fig. 2. Distribution of colloidal gold particles in the localization of RuBisCO and PRK in *C. fritschii* by immunoelectronmicroscopy. (a) RuBisCO distribution: 76 individual cells examined; 9345 gold particles counted. (b) PRK distribution: 45 individual cells examined; 28853 gold particles counted. In control experiments, the number of gold particles on cell sections treated with null serum plus gold-goat anti-rabbit serum, and with gold-goat anti-rabbit serum alone, varied between zero and 0.6% of the numbers of particles associated with antiserum treated sections. CA, carboxysomes; CY, cytoplasm; M, cytoplasmic membrane; O, all other discernible inclusion bodies; N, labelling on outer layers external to cytoplasmic membrane; marker bars represent SD.

on a suitable concentration of appropriate antiserum for 18 h at 4 °C. Antisera against purified RuBisCO from *Microcystis 7820* and PRK from *C. fritschii* were raised in rabbits as before and shown to be monospecific according to rocket immunoelectrophoresis (Lanaras & Codd, 1981b). The grids were then washed gently in tap water and 0.02% (w/v) KCl for 15 min before incubating for 1 h with 20 nm colloidal gold probe conjugated to goat anti-rabbit IgG (GAR G20, Jensen Pharmaceutica, Beerse, Belgium) to a dilution of 1/15. Preparations were dried in air and negatively stained using 0.2% (w/v) osmium vapour, and examined using an Associated Electrical Industries EM 801 transmission electron microscope.

RESULTS AND DISCUSSION

Typical results of RuBisCO protein localization using RuBisCO antiserum, raised in rabbits, followed by colloidal gold-conjugated goat anti-rabbit serum, are shown in Fig. 1(a). The label was consistently clustered over the sectioned carboxysomes, but was not significantly associated with other inclusion bodies. RuBisCO antiserum labelling of the cytoplasm also occurred, confirming *in vitro* findings that part of the cyanobacterial cell complement of RuBisCO is carboxysomal and that the remainder is cytoplasmic (Codd & Stewart, 1976; Lanaras & Codd, 1981a, b, 1982). Immunocytochemical labelling of the cyanobacterium *Anabaena cylindrica* using RuBisCo antiserum has also resulted in labelling of the carboxysomes, and of the cytoplasm, where label was present in the region of the thylakoids (Cossar et al., 1985). The distribution of PRK antiserum labelling was markedly different from that of RuBisCO. Carboxysomes were not significantly labelled (Fig. 1b); most of the gold particles were observed in the cytoplasm and in the peripheral region inside the cytoplasmic membrane. The percentage distribution of gold particles in the presence of both antisera, as determined in sections of whole *C. fritschii* cells harvested from early stationary phase photoautotrophic batch culture, is given in Fig. 2.

The present data confirm that the *C. fritschii* carboxysomes are a site of RuBisCO accumulation and that the inclusions do not contain PRK (Lanaras & Codd, 1981a; Marsden et al., 1984). We have obtained similar results by the immunogold labelling approach in cell sections of the unicellular cyanobacterium *Anacystis nidulans* R2 (*Synechococcus PCC 7942*) (unpublished data), indicating a similar composition between carboxysomes from filamentous and unicellular strains, despite considerable variation in carboxysome size, shape and abundance within cyanobacteria (Codd & Marsden, 1984).

We thank the Nuffield Foundation for financial support and Dr W. J. N. Marsden for help with techniques. A.M.H. thanks the Science and Engineering Research Council for a postgraduate studentship.
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