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A cytoplasmic-membrane protein repressible by ammonium in *Synechococcus* R2: altered expression in nitrate-assimilation mutants

Francisco Madueño, Miguel A. Vega-Palas, Enrique Flores and Antonia Herrero

Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y CSIC, Apdo 1113, 41080-Sevilla, Spain

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A protein of ~48 kDa was present in the cytoplasmic membrane of cells of *Synechococcus* R2 grown on nitrate or incubated in the absence of combined nitrogen, but not in ammonium-grown cells. The level of this polypeptide was diminished in mutant strains that also exhibit reduced levels of other components of the nitrate-reductive system. On the other hand, a mutant constitutive for nitrate uptake also synthesized constitutively the 48 kDa protein.

Membrane protein; Ammonium repression; Nitrate assimilation; (Cyanobacterium)

1. INTRODUCTION

Nitrate represents an excellent nitrogen source for the growth of cyanobacteria. Nitrate assimilation involves nitrate uptake into the cell and its reduction, via nitrite, to ammonium, which is the inorganic nitrogen form incorporated into carbon skeletons [1]. Nitrate reductase, a molybdenum-containing enzyme, and nitrite reductase have been isolated from some cyanobacteria, representing single-polypeptide enzymes of about 75 and 50 kDa, respectively [1]. No information is currently available about any other protein component of the nitrate-assimilation system, which might include, for instance, proteins involved in the uptake and metabolism of molybdenum and in the uptake of nitrate. Although, from physiological evidence, the existence in cyanobacteria of an active transport of nitrate has been inferred [2–5], the presumptive permease of nitrate has not been identified to date.

Correspondence address: E. Flores, Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Facultad de Biología, Apdo. 1113, 41080-Sevilla, Spain

We are interested in defining the different elements involved in nitrate assimilation in cyanobacteria. Since physical separation of the cell wall and of the cytoplasmic membrane from the intracellular photosynthetic membranes is now possible with several cyanobacteria [6,7], we have used this approach to study, in *Synechococcus* R2, the cytoplasmic-membrane protein(s) associated with growth on nitrate.

2. MATERIALS AND METHODS

Several strains derived from the unicellular cyanobacterium *Synechoccocus* R2 (PCC 7942) were used in this work. Strain A6 carries transposon Tn901 in plasmid pUH24, and strain M4$^-$ lacks this plasmid. Strain FM2 is a nitrate-assimilation defective mutant derived from strain A6 that lacks nitrite reductase and expresses constitutively both nitrate reductase [8] and the ability to take up nitrate [9]. Strains CS1 and CS13 are mutants derived from strain M4$^-$ impaired in the synthesis of nitrate reductase, nitrite reductase and glutamine synthetase, whose detailed characterization will be described elsewhere (Vega-Palas, M.A., Flores, E. and Herrero, A., unpublished). CS1.1 and CS13.1 are revertants from CS1 and CS13, respectively, that regained wild-type expression of nitrate reductase, nitrite reductase and glutamine synthetase.

Cells were grown photoautotrophically at 39°C with nitrate or ammonium as the nitrogen source, as described [10]. Derepression of ammonium-grown cells was carried out by in-

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cubation under culture conditions in the absence of any source of nitrogen for about 18 h.

For the isolation of cytoplasmic-membrane fractions, cells were disrupted and fractionated as described by Omata and Ogawa [11]. Essentially, 0.5–1 g (wet wt) of cells were treated with lysozyme and disrupted in a French press. After the addition of DNase and inhibitors of proteases, and retiring unbroken cells by low-speed centrifugation, the cell-free extract was subject to high-speed centrifugation (130000 × *g*, 17 h) in a discontinuous sucrose gradient. Cytoplasmic membranes, that formed a band in the 30% sucrose layer, were subsequently pelleted by centrifugation at 160000 × *g* for 2 h (after dilution of the preparation to less than 6% sucrose), resuspended in 0.1–0.2 ml of 10 mM Tes-NaOH buffer (pH 7) containing 10 mM NaCl and 5 mM EDTA, and their protein content determined [12]. Samples of cytoplasmic membranes containing 20–30 μg protein were subjected to SDS-PAGE on 12% gels. The samples were mixed with an equal volume of sample buffer [13] and incubated in boiling water for 4 min before being applied to the gels. SDS-PAGE was carried out as in [13] and the proteins were visualized with Coomassie brilliant blue R-250.

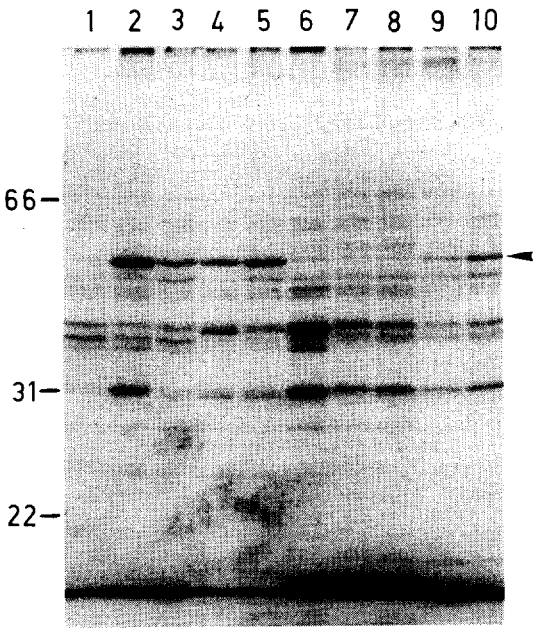

Fig.1. Cytoplasmic-membrane proteins in *Synechococcus* sp. Lanes contained proteins from the following: (1) strain A6, ammonium-grown; (2) strain A6, nitrate-grown; (3) strain A6, derepressed in N-free medium; (4) strain FM2, ammonium-grown; (5) strain FM2, derepressed in N-free medium; (6) strain CS13, ammonium-grown; (7) strain CS13, derepressed in N-free medium; (8) strain CS1, derepressed in N-free medium; (9) strain CS13.1, derepressed in N-free medium; and (10) strain CS1.1, derepressed in N-free medium. Similar patterns of proteins to those obtained with strain A6 were also obtained with strain M4$^-$. Arrowhead points to the polypeptide of about 48 kDa. The sizes of three molecular mass markers (kDa) are shown on the left margin.

3. RESULTS AND DISCUSSION

Cytoplasmic membranes from wild-type *Synechococcus* grown on nitrate were repetitively observed to contain a protein of ~48 kDa that was missing from (or much decreased in) ammonium-grown cells (fig.1, lanes 1–2). Because this protein was also observed after derepression of ammonium-grown cells in the absence of any nitrogen source (fig.1, lane 3), it appears to be repressible by ammonium rather than inducible by nitrate. An ammonium-repressible protein of similar molecular mass has also been observed in cytoplasmic-membrane preparations of another unicellular cyanobacterium [M.N. Sivak, et al. (1988) III Congreso Luso-Español de Bioquímica, Santiago de Compostela]. It has been shown [5] that nitrate-grown, but not ammonium-grown, cells of *Synechococcus* are able to transport nitrate. The nitrate-reduction system of unicellular cyanobacteria is also repressible by ammonium rather than inducible by nitrate [1,10], but it has been localized in the photosynthetic membranes of these organisms [14,15]. It is of interest that cytoplasmic-membrane proteins repressible by CO_2 [11] or sulfate [16] have also been reported in *Synechococcus*.

Mutants CS1 and CS13 are impaired in the expression of several enzymes involved in nitrogen assimilation. As shown in fig.1 (lanes 6–8), mutants CS1 and CS13 exhibited low levels of the 48 kDa protein even after incubation in the absence of any nitrogenous supplement. Revertants from these mutants (strain CS1.1 and CS13.1, respectively) did express, as the wild-type strain, high levels of the 48 kDa cytoplasmic-membrane protein in nitrogen-free medium (fig.1, lanes 9–10) and low levels in medium containing ammonium (not shown). These results suggest that the synthesis of that protein is under the control of a system that regulates nitrogen assimilation.

Strain FM2 is a pleiotropic mutant that constitutively expresses nitrate reductase [8] and the ability to take up nitrate [9]. Cytoplasmic membranes from mutant FM2 exhibited the 48 kDa protein after growth on ammonium as well as after incubation in nitrogen-free medium (fig.1, lanes

4–5). Constitutive expression in strain FM2 is what would be expected for a protein required for the transport of nitrate.

The present results suggest that the 48 kDa protein of the cytoplasmic membrane represents a transport component of the nitrate assimilation system of *Synechococcus* R2, an interesting possibility being that it corresponds to the nitrate permease.

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