SHORT COMMUNICATION

Metabolic Activities of Akinetes of the Cyanobacterium *Anabaena doliolum*: Oxygen Exchange, Photosynthetic Pigments and Enzymes of Nitrogen Metabolism

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(Received 30 January 1984)

All vegetative cells of *Anabaena doliolum* differentiated into akinetes. These showed respiratory O₂ uptake but lacked photosynthetic pigments and O₂ evolution. Activities of nitrogenase, glutamine synthetase, nitrate reductase and aspartate dehydrogenase were totally absent, while glutamate dehydrogenase showed extremely low activity. Glutamate-oxaloacetate transaminase activity was similar to that in whole filaments but alanine dehydrogenase and glutamate-pyruvate transaminase activities were much lower. Thus, transformation of vegetative cells into akinetes was accompanied by the loss of photosynthetic machinery, ammonia-generating systems and the enzyme of primary ammonia assimilation.

INTRODUCTION

Filamentous cyanobacteria show a maximum of three cell types: vegetative cells, heterocysts and akinetes (spores). The latter are resistant to various external factors and have undergone morphological, physiological and macromolecular changes during their development from vegetative cells (see Nichols & Adams, 1982). In general the akinetes have large amounts of carbon and nitrogen storage products (Miller & Lang, 1968; Sarma & Kantu, 1979; Sutherland *et al.*, 1979), a lower lipid content (Yamamoto, 1972) and lower activities of photosynthesis and CO₂-fixation (Fay, 1969*a*; Yamamoto, 1976) and of respiration (Chauvat *et al.*, 1982; however, see Fay, 1969*a*). At least in some cyanobacteria heterocysts are involved in differentiation of akinetes from vegetative cells (Wolk, 1966; Sutherland *et al.*, 1979).

There is no information regarding the enzymes of nitrogen metabolism in akinetes and the data on their photosynthetic pigment composition are sporadic and inconsistent (Fay, 1969*b*; Wolk & Simon, 1969; Singh & Sunita, 1974; Sutherland *et al.*, 1979; Chauvat *et al.*, 1982; see also Nichols & Adams, 1982). We have studied these aspects in akinetes of *Anabaena doliolum* and present evidence to show that they are functionally deficient in both photosynthesis and enzymes of inorganic nitrogen metabolism.

METHODS

Organism. Axenic batch cultures of *Anabaena doliolum* were grown in modified Chu-10 medium (Gerloff *et al.*, 1950) at 28 °C and at a photon fluence rate of 50 µmol m⁻² s⁻¹. Akinetes were formed after two weeks, at the end

Abbreviations: ADH, alanine dehydrogenase; ASDH, aspartate dehydrogenase; GDH, glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; GS, glutamine synthetase; NR, nitrate reductase.

of growth. The source of the *A. doliolum* strain and its isolation and purification have been described (Singh & Singh, 1964).

Light microscopy. Light micrographs were taken using a Leitz photomicroscope.

Protein estimation. The Lowry method was used with bovine serum albumin as standard.

Pigment content. The *in vivo* pigment compositions were analysed by using a photoacoustic spectrometer model 6001 (E.G. & G. Princeton Applied Research Corp., USA) scanning from 500 to 700 nm at room temperature with 40 Hz modulation frequency (Balasubramanian & Rao, 1982). Chlorophyll *a* estimation was also done by using the method of Mackinney (1941).

Oxygen exchange. Photosynthetic and respiratory activities of whole filaments and akinetes were measured in a polarographic Clark electrode. A 3 ml cell suspension containing 1 to 2 mg protein ml⁻¹ was placed in the reaction chamber and maintained at 28 °C. Photosynthesis was measured as O₂ evolution under saturating white light and respiration as O₂ consumption in the dark.

Enzyme assays. All the assays were done in cell-free extracts except nitrogenase, which was assayed in whole cells. Nitrogenase activity was assayed according to Stewart *et al.* (1967), glutamine synthetase (EC 6.3.1.2 and 2.3.2.2) according to Sampaio *et al.* (1979), aspartate dehydrogenase (EC 1.4.1.5) according to Haystead *et al.* (1973) and glutamate-pyruvate transaminase (EC 2.6.1.2) according to Jäger & Weigel (1978). Glutamate dehydrogenase (EC 1.4.1.2 and 1.4.1.4), alanine dehydrogenase (EC 1.4.1.1) and glutamate-oxaloacetate transaminase (EC 2.6.1.1) were measured as described by Stewart & Rowell (1977) and nitrate reductase (EC 1.7.99.4) as described by Manzano *et al.* (1976).

Chemicals. All enzymes and enzyme substrates were purchased from Sigma and other chemicals from BDH.

RESULTS AND DISCUSSION

Since all the vegetative cells were transformed into akinetes at the end of growth and sporulation was simultaneous (see also Singh & Srivastava, 1968), a pure and uniform population of akinetes was obtained. Such akinetes showed a respiration rate of 48.6 nmol O₂ uptake min⁻¹ (mg protein)⁻¹ as compared to 79.1 in whole filaments, indicating a lower rate of respiration, as found in the case of *Nostoc* PCC 7524 (Chauvat *et al.*, 1982).

Photosynthetic O₂ evolution was absent in akinetes although whole filaments showed a rate of 343 nmol O₂ evolved min⁻¹ (mg protein)⁻¹. This, together with the photoacoustic spectra showing the lack of photosynthetic pigments (Fig. 1) and the lack of photosynthetic pigments in akinete extracts of *A. doliolum* reported earlier (Singh & Sunita, 1974), provides conclusive evidence that the akinetes are non-photosynthetic. Apart from analysing photosynthetic pigments using photoacoustic spectroscopy, we have also extracted these pigments in methanol after breaking akinetes in liquid nitrogen. We did not find any chlorophyll *a* peak in the methanol extract. Singh & Sunita (1974) reported that chlorophyll *a* was absent in dark spores which were more synchronized than the light spores. The latter were reported to have low amounts of chlorophyll *a*. Possibly, the low chlorophyll content detected in light spores may have been due to spores of different age in the population, some of which may have started chlorophyll synthesis to be followed by germination. Akinetes of *A. doliolum* are able to germinate in non-photosynthetic light (Kaushik & Kumar, 1970) and the role of light in the germination process may not be through photosynthesis but could be through a photomorphogenetic process analogous to phytochrome (Kaushik & Kumar, 1970; Singh & Sunita, 1974; Reddy *et al.*, 1975; Braune, 1979).

The activities of various nitrogen-metabolizing enzymes are presented in Table 1. The lack of nitrogenase activity is consistent with the earlier finding for *A. cylindrica* akinetes (Fay, 1969*a*). GS (both biosynthetic and transferase), ASDH and NR activities were undetectable in akinetes, although whole filaments showed normal activities. GDH, ADH and GPT activities in akinetes were, respectively, only 13%, 27% and 36% of those in whole filaments; however, GOT activity was similar in both.

Thus, our data show that *A. doliolum* akinetes are non-photosynthetic and lack activities of the enzymes of inorganic nitrogen metabolism. These akinetes differ from those of *A. cylindrica* (Wolk & Simon, 1969), *A. variabilis* (Braune, 1979) and *Nostoc* PCC 7524 (Sutherland *et al.*, 1979), which contain photosynthetic pigments; however, *Nostoc* PCC 7524 akinetes show a photosynthetic rate which is only 10% of that in filaments (Chauvat *et al.*, 1982). The

Fig. 1. Photosynthetic spectra of (a) whole filaments and (b) intact akinetes. Peaks 1, 2 and 3 correspond to phycoerythrin, phycocyanin and chlorophyll *a*, respectively.

Table 1. Activities of nitrogen-metabolizing enzymes in cell-free extracts of *A. doliolum* and its akinetes

Whole filaments were harvested from exponential-phase cultures. Akinetes and whole filaments were repeatedly passed through a French pressure cell at 110 MPa for preparation of cell-free extracts (over 95% of the cells were broken). The data are means of duplicate assays, which did not vary by more than 10%.

Enzyme	Activity [nmol product formed min ⁻¹ (mg protein) ⁻¹]	
	Akinetes	Whole filaments
ADH	<1.49	5.53
ASDH	ND	15.5
GDH (NADH-dependent)	ND	ND
GDH (NADPH-dependent)	<1.99	15.47
GOT	9.96	9.94
GPT	4.96	13.84
GS (biosynthetic)	ND	77.4
GS (transferase)	ND	1580
NR	ND	6.48
Nitrogenase	ND	9.97*

ND, Not detectable.

* nmol C₂H₄ produced (μg chlorophyll *a*)⁻¹ h⁻¹.

disappearance of nitrogen-metabolizing enzymes (e.g. GS) could be used as a marker of akinete development and maturation.

The total lack of photosynthetic machinery, nitrogenase activity, enzymes of ammonia generation from inorganic nitrogen sources and the primary ammonia assimilating enzyme makes *A. doliolum* akinetes an ideal model for the study of regulatory and developmental aspects of photosynthesis, the heterocyst-nitrogenase system and enzymes of inorganic nitrogen metabolism.

Research support from INSA is thankfully acknowledged. V. V. Rao thanks CSIR for a research scholarship.

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