Role of intracellular carbonic anhydrase in inorganic-carbon assimilation by Porphyridium purpureum

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Abstract. Air-grown cells of Porphyridium purpureum contain appreciable carbonic-anhydrase activity, comparable to that in air-grown Chlamydomonas reinhardtii, but activity is repressed in CO2-grown cells. Assay of carbonic-anhydrase activity in intact cells and cell extracts shows all activity to be intracellular in Porphyridium. Measurement of inorganic-carbon-dependent photosynthetic O2 evolution shows that sodium ions increase the affinity of Porphyridium cells for HCO3-. Acetazolamide and ethoxyzolamide were potent inhibitors of carbonic anhydrase in cell extracts but at pH 5.0 both acetazolamide and ethoxyzolamide had little effect upon the concentration of inorganic carbon required for the half-maximal rate of photosynthetic O2 evolution (K0.5[CO2]). At pH 8.0, where HCO3- is the predominant species of inorganic carbon, the K0.5(CO2) was increased from 50 μM to 950 μM in the presence of ethoxyzolamide. It is concluded that in air-grown cells of Porphyridium, HCO3- is transported across the plasmalemma and intracellular carbonic anhydrase increases the steady-state flux of CO2 from inside the plasmalemma to ribulose-1,5-bisphosphate carboxylase-oxygenase by catalysing the interconversion of HCO3- and CO2 within the cell.

Key words: Carbonic anhydrase (intracellular) – Inorganic carbon assimilation – Porphyridium (carbonic anhydrase).

Introduction

Unicellular green microalgae, such as Chlamydomonas reinhardtii when grown with air-levels of CO2 show an increased affinity for CO2 compared with cells that have been grown on air enriched with 1 to 5% (v/v) CO2 (Findenegg 1976; Hogetsu and Miyachi 1977; Badger et al. 1980; Moroney and Tolbert 1985). This increased affinity for external inorganic carbon seems to be correlated with the ability of air-grown microalgae to transport and accumulate inorganic carbon internally to higher levels than the external concentration (Badger et al. 1980; Beardall 1981; Beardall and Raven 1981; Coleman and Colman 1981; Kaplan et al. 1982; Spalding and Ogren 1983; Shelp and Canvin 1985; Moroney et al. 1985; Patel and Merrett 1986a). Adaptation to growth on air-levels of CO2 is accompanied by the derepression of carbonic anhydrase (Nelson et al. 1969; Findenegg 1976; Coleman et al. 1984) leading to the concept that the increased affinity of air-grown cells for external carbon is the result of a bicarbonate pump. However, in air-grown cells of Chlamydomonas much of the carbonic anhydrase is present in the periplasmic space (Kimpel et al. 1983) and it has been proposed that CO2 not HCO3- is the species that diffuses across the plasmalemma (Imamura et al. 1983; Moroney and Tolbert 1985; Patel and Merrett 1986a). Carbonic anhydrase external to the plasmaemla maintains the CO2 supply by catalysing the conversion of HCO3- to CO2 (Tsuzuki 1983; Moroney et al. 1985).

Unlike Chlamydomonas some marine microalgae lack carbonic anhydrase external to the plasmalemma and HCO3- is the inorganic-carbon species that is translocated across the plasmalemma (Patel and Merrett 1986b). Porphyridium purpureum is a soil alga that grows best in marine liquid media, indicating that it is probably of brackish or marine origin (Lee 1980). In this paper we report on the role of intracellular carbonic anhydrase in inorganic-carbon assimilation by this alga.
Material and methods

Growth of cells. Axenic cultures of Porphyridium purpureum (Cambridge Culture Collection of Algae and Protozoa, Cambridge, UK; strain 1380/1A) were grown at pH 8.0 on a medium based on ASP-2 (Provasoli et al. 1957). Cultures were grown in 500-ml dreschel bottles aerated with air, at 20°C with a photon flux of 80 μmol m⁻² s⁻¹ at the culture surface, provided by "cool-white" fluorescent lamps. Cultures were harvested in the late exponential phase of growth.

Determination of carbonic anhydrase (EC 4.2.1.1) activity. Cells were harvested by centrifugation at 400 g, washed twice and resuspended in 25 mM Veronal buffer pH 8.2 and carbonic-anhydrase activity measured by a modification of the electrophoretic method of Wilbur and Anderson (1948). The reaction mixture contained 5 ml 25 mM Veronal buffer pH 8.2 and up to 2.0 ml of cell suspension or enzyme solution at 3°C. Saturated CO₂ solution (4 ml) was injected into the mixture by syringe and the decrease in pH followed with time. For the uncatalysed reaction the enzyme solution was substituted by the same volume of buffer. Enzyme units were calculated from the time taken to lower the pH from 8.2 to 7.2 using the formula:

\[ E.U. = \frac{10^6}{t_e - t_u} \]

where \( t_u \) and \( t_e \) are the times of the uncatalysed and enzyme-catalysed reactions, respectively.

Preparation of cell extracts. Cells were harvested by centrifugation at 4000 g, washed once with 25 mM Veronal buffer pH 8.2 and resuspended in 500 mM mannitol, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) pH 8.0 to a density of 1·10⁸ cells ml⁻¹. Cells were disrupted by passage through a French pressure cell at 100 mPa and extracts clarified by centrifugation at 20000 g for 30 min. The supernatant was assayed for carbonic-anhydrase activity.

Inorganic-carbon-dependent O₂ evolution. Cells were harvested by centrifugation at 4000 g, washed twice and resuspended in 500 mM mannitol, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) pH 8.0 to a density of 1·10⁸ cells ml⁻¹. Prior to the addition of cells the buffer was bubbled with N₂ to decrease the dissolved CO₂ and O₂. Cells were kept on ice in darkness until required. Oxygen evolution was measured using a Clark-type oxygen electrode (Hansatech, King's Lynn, Norfolk, UK). A 1 ml suspension was incubated at 20°C with a photon flux of 500 μmol m⁻² s⁻¹. (Ellipsoid Halogen reflector bulb; Philips, Eindhoven, The Netherland). The chamber was closed and the cells allowed to deplete endogenous carbon sources as measured by the cessation of O₂ evolution. The rate of O₂ evolution was then determined following the addition of varying concentrations of KHCO₃.

Other methods. Protein in cell extracts was estimated by the modified Lowry method described by Hartree (1972). Cell numbers were determined using a haemocytometer. K constants were derived from experimental data after the linear transformation of Woolf (1932).

Results

Carbonic-anhydrase activity. Many freshwater algae when grown on air-levels of CO₂ show high levels of carbonic-anhydrase activity but when cells are grown with air enriched with 1–5% CO₂ carbonic-anhydrase activity is repressed. With Porphyridium grown at pH 8.0 at a high CO₂ concentration (5% v/v CO₂) carbonic-anhydrase activity is minimal (Fig. 1). Transferring high-CO₂ cells to air levels of CO₂ (0.03% CO₂, v/v) resulted in the gradual derepression of carbonic-anhydrase activity over a 24-h period (Fig. 1). The final level of carbonic-anhydrase activity attained in air-grown cells of Porphyridium is similar to that found in air-grown Chlamydomonas reinhardtii (Table 1). Measurement of carbonic-anhydrase activity in intact cells and cell extracts shows that this activity is contained within the plasmalemma (Fig. 1, Table 1). In this respect, Porphyridium is similar to the marine algae Skeletonema costatum and Phaeodacty-