Influence of inorganic Phosphorus on phosphatase activity in stationary phase culture of *Nannochloropsis gaditana*

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Some microalgae species are able to grow with glycerophosphate as the only source of phosphorus (LUBIAN, 1981), which implies the presence of phosphatases in the algae. This presence has been widely studied in both freshwater and marine microalgae (KUENZLER & PERRAS, 1965; LIEN & KNUTSEN, 1973; CARPENE & WYNNE, 1986). The phosphatase activity was also detected in *Nannochloropsis gaditana*, this microalgae show only a maximum at pH 5.5 for whole cell and soluble and particulate fraction (LUBIAN *et al.*, 1992). The regulatory role of phosphorus in the synthesis of acid and alkaline phosphatases was shown for several algal species (KNUTSEN, 1968; VAN BOEKEL & VELDHUIS, 1990). In this paper, we have studied the effect of inorganic phosphorus on phosphatase activity in stationary phase culture of *Nannochloropsis gaditana*.

Continuous culture of *Nannochloropsis gaditana* was carried out in a reactor of 1.5 l with f/2 medium enriched (GUILLARD & RYTHER, 1962), natural sea-water (36‰ salinity) containing a double amount of nitrate and phosphate at constant temperature of 20°C and continuous lighting (100 $\mu E.m^{-2}.s^{-1}$) with day light fluorescent lamps. When the phosphorus was omitted from medium the composition of this was the same but without phosphate.

Phosphatase activity was estimated by the liberation of p-nitrophenol from p-nitrophenyl ortophosphate (disodium salt, Merck) at saturating concentrations. Buffer was 100 mM citric acid/sodium citrate. When analyzing whole cell, buffer was made up with sea-water. For whole cell phosphatase activity, the reaction mixture contained 0.5 ml buffer, 0.5 ml microalgae culture and 50 µl substrate. Final concentration was 20 mM. For cell fractioning, the culture was centrifuged at 1600 g for 15 minutes, the supernatant discharged and the pellet washed twice in 0.6 M NaCl, 2 ml of 100 mM Tris-HCl at pH 7.0 were finally added and sonicated for 20 minutes in two steps. The extract was centrifuged for 15 minutes at 1600 g and the supernatant employed as enzyme source for the determination of phosphatase activity in soluble fraction. To the pellet resulting from the second centrifugation was added 2 ml of 100 mM Tris-HCl at pH 7.0 and sonicated for 5 minutes to resuspend it. The reaction mixture contained 1.0 ml buffer, 0.1 ml substrate and 0.1 ml extract or 0.1 ml of suspension depending on whether the soluble or the particulate fraction was analyzed. Final concentration was 50 mM. For all determinations of phosphatase activity a blank was run in parallel. The assay temperature was 30° C and incubation time was 60 minutes. The reaction was stopped by adding 100 mM NaOH to the reaction mixture. Phosphatase activities were calculated using a molar extinction coefficient of 18.5 cm^2. µmol^{-1} at 405 nm (WALTER & SCHUTT, 1974). Results are expressed as U.(10^{12}cell)$^{-1}$ (1U=1µmol p-nitrophenol. min^{-1}). For determining of cell phosphorus, 15 ml of culture was centrifuged at 1600 g for 15 minutes, and the pellet resulting was washed with 0.6 M NaCl twice. The pellet was digested with potassium persulphate at 130°C for 60 minutes. Inorganic phosphorus was determined with an autoanalyzer Technicon TRAACS 800. The analytical method was a modification of GRASHOFF *et al.*, (1983).

Cell density showed a weakly increase in the period 0-72.5 hours (range 19.0-28.0 10^6 cell. ml^{-1})(Fig. 1A), thus inorganic phosphorus flux was eliminated at 25.5 hours. For this reason, a fast decrease was observed for it (15.6 µg-at P.l^{-1} at 25.0 hours and 0.09 µg-at P.l^{-1} at 195.5 hours)(Fig 1B). Thus at 193 hours an inorganic flux was newly added, inorganic phosphorus concentration only showed a little increase (2.5 µg-at P.l^{-1}). Notwithstanding cell phosphorus was increased at 1.8 µg-at P.l^{-1} (Fig. 1A). Whole cell phosphatase activity (Fig. 1B) showed an increasing with time, ranging between 200 U.(10^{12} cell)$^{-1}$ at initial period (0-72.5 hours) and 450 U.(10^{12} cell)$^{-1}$ at last period (192-245 hours). A similar evolution for soluble fraction phosphatase activity was observed. In the case of particulate fraction a decrease was observed at final period.

Increase of whole cell phosphatase activity and the decrease of external inorganic phosphorus may reflect a dependence relation between them. The role of cell phosphorus in the regulation of phosphatase synthesis is likely important, however a gap period was observed in the response of phosphatase activity at the variation of cell phosphorus.

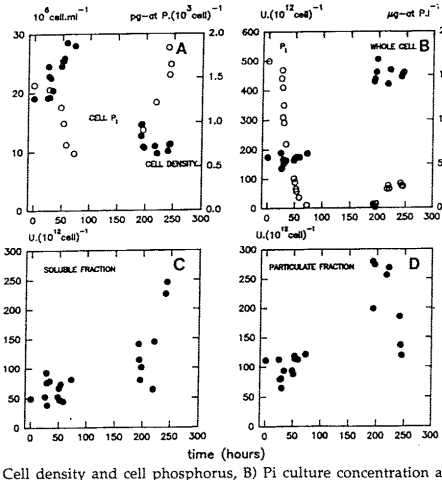

Fig. 1.- A) Cell density and cell phosphorus, B) Pi culture concentration and whole cell phosphatase activity C) and D) soluble and particulate fraction phosphatase activity, respectively.

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