INFLUENCE OF β-GLYCEROPHOSPHATE, ACETATE AND CO_2 IN THE APPEARANCE OF AN ALKALINE PHOSPHATASE IN *Chlamydomonas reinhardii*

A. M. Guerrini*, T. Cremona and E. C. Preddie**
International Institute of Genetics and Biophysics,
Naples, Italy

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SUMMARY: Cultures of Chlamydomonas r. do not express the gene for alkaline phosphatase when inorganic phosphate is in the medium; however, the enzyme is present when β-glycerophosphate is added. The carbon source plays an important role in the synthesis of the phosphatase; in fact, the acetate functions as a co-inducer of a series of structural genes.

The green algae *Euglena gracilis*, *Chlamydomonas mundana* and *Chlamydomonas r.* synthesize two distinct aldolases (1-4). Russell and Gibbs showed that when a culture of *Chlamydomonas mundana* is shifted from CO_2 to acetate as a carbon source, Aldolase II replaces Aldolase 1 (5). In *Chlamydomonas r.* the situation is different because it is possible to synthesize either Aldolase I or both proteins (6). In fact, algae grown 48 hours, with alternating periods of light and dark, in the presence of a mixture of air and 5% CO_2 contain only Aldolase I. However, the same culture after five days contains both enzymes, despite the fact that the carbon source has not been changed (6).

The discovery that carbon source and metabolic conditions may regulate the synthesis of at least two proteins in *Chlamydomonas r.* led us to study another enzyme, alkaline phosphatase, to see if it behaved in a similar way. This paper demonstrates the existence of an alkaline

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*Recipient of an E.M.B.O. Fellowship, 1968-1970.
**Present Address: Department of Biological Sciences
University of Montreal, Montreal, Canada.

phosphatase in Chlamydomonas r. and describes the mode of appearance of this protein under various growth conditions.

MATERIALS AND METHODS

Growth medium was prepared according to Sager and Granick (7); the illumination was 400-600 foot candles, temperature 25°. Chlamydomonas r. was a kind gift from Dr. R. Sager. Streptomycin sulfate was purchased from Lepetit S.P.A., Milano, and β-glycerophosphate was purchased from Sigma Co., St. Louis, Mo. Cells were broken with a Sonifier cell disruptor, Model W 140D, Heat System Ultrasonic, Inc., Melville, N. Y. and enzymatic activity was measured in a Gilford Spectrophotometer Model 2000.

Assays for alkaline phosphatase (8) were carried out in the following way: aliquots of 10^8 cells were collected by centrifugation, resuspended in 10 ml of 0.05M Tris-HCl, pH 7.5 and sonicated at 0-4° for 1 minute. The yield of disrupted cells was about 75-80%. The crude extract was clarified by centrifugation and by Streptomycin precipitation and then tested for presence of alkaline phosphatase using p-nitrophenylphosphate at pH 8.5 as substrate. Even with cultures containing young, small cells, alkaline phosphatase was usually detected under these conditions by adding to the substrate amounts of extract varying from 2 to 50 l. When no positive response was detected in this range, the amount of added extract was increased to 100 or 200 l.

RESULTS AND DISCUSSION

When cultures of Chlaymdomonas r., growing logarithmically in the presence of air and 5% CO_2 in a routine medium containing inorganic phosphate, were transferred into a medium where the phosphate required for growth had been substituted with the same quantity of β-glycerophosphate, a diauxy-type phenomenon (9) was observed (Fig. 1b). After a residual exponential growth, a 24 hour lag period was observed, after which the cells entered a logarithmic phase, the rate of which was related to the concentration of the β-glycerophosphate. Subcultures of

these cells in a fresh β-glycerophosphate medium continued to grow without showing any diauxy-type phenomenon (Fig. 1c).

Assays for alkaline phosphatase showed that no activity was detectable in the control culture (Fig. 1a); enzyme activity, however, was found at 48 hours, (about four generation-times), after transfer into glycerophosphate (Fig. 1b), and, of course, in the subculture as described in Fig. 1c.

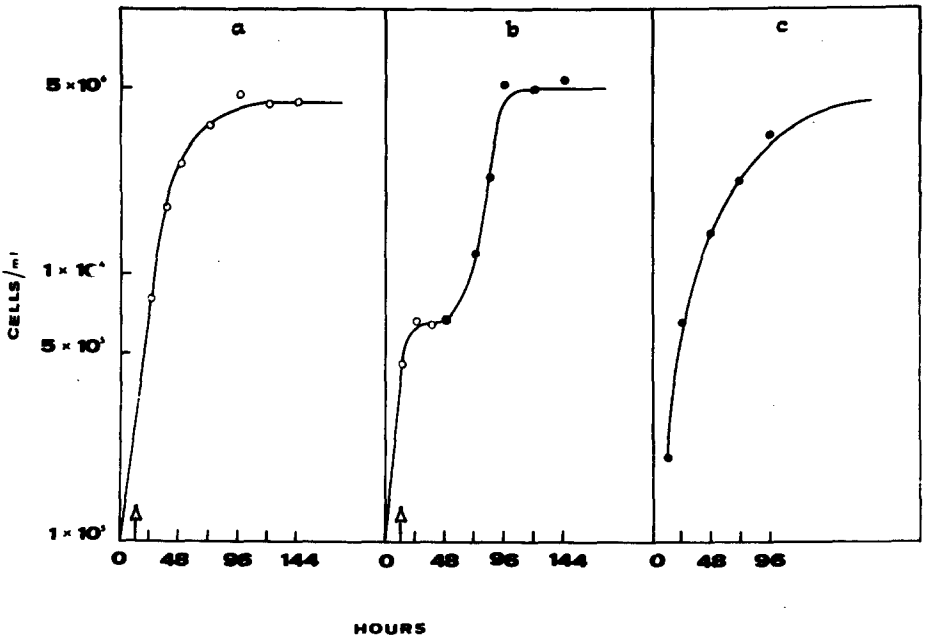

Fig. 1. Growth profiles of Chlamydomonas r.: An aliquot of cells growing at logarithmic rate was collected and washed three times by centrifugation and then inoculated into fresh media at the concentration of approximately 1 x 10^5 cell/ml. 1a) Control curve: medium containing 200 mg of inorganic phosphate according to ref. (7). 1b) Same as before but equal molar amount of phosphate as sodium β-glycerophosphate has been added (470 mg/l) instead of the inorganic phosphate. 1c) An aliquot taken from the culture 1b growing at exponential rate (96 hours) was placed in a fresh medium containing β-glycerophosphate.
In addition to the number of cells/ml, closed and opened circles indicate respectively presence or absence of alkaline phosphatase. Closed and open arrows indicate respectively presence or absence of alkaline phosphatase in the early cultures in which a correct determination of number of cells was not carried out.

These data suggest that, under standard growth conditions with inorganic phosphate-containing medium, the phosphatase gene is not expressed. In this phenomenon, which has been shown to occur in many other organisms, the β-glycerophosphate appears to act as an inducer of phosphatase synthesis, thereby permitting utilization of the β-glycerophosphate.

Fig. 2b describes the growth of a culture in a medium (7) containing 0.3% sodium acetate instead of a mixture of air and 5% CO_2. When, in this culture, inorganic phosphate was replaced with β-glycerophosphate, the growth curve (Fig. 2b), which extrapolates to time zero, indicating that there was not a detectable lag in cell division, did not show any diauxy-type phenomenon; and, furthermore, the alkaline phosphatase was

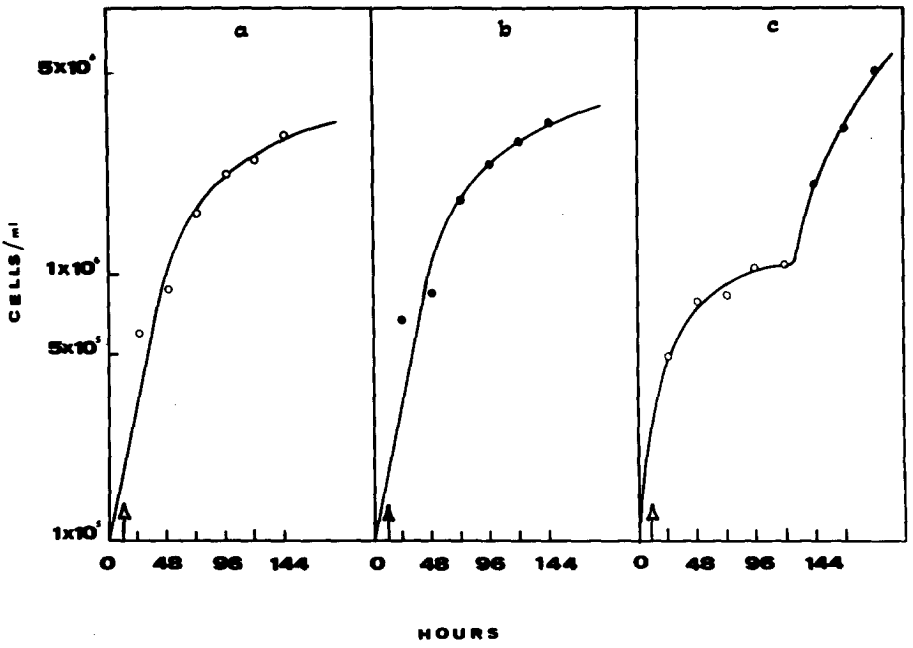

Fig. 2. Growth of Chlamydomonas r. in acetate: Same conditions as in the previous figure but no aeration with a mixture of air and CO_2 was performed and 3g/l of sodium acetate were added instead. 2a) Control curve: medium containing 200 mg/l inorganic phosphate. 2b) Same as in 2a but the inorganic phosphate has been replaced by equal molar amount of β-glycerophosphate (470 mg/l). 2c) Culture grown in presence of acetate (3 g/l); β-glycerophosphate (470 mg/l); 2 mg/l inorganic phosphate. Symbols as in Fig. 1.

present at the earliest time measured (12 h).

Fig. 2c describes the growth of a culture in the presence of acetate, β-glycerophosphate and an amount of inorganic phosphate (2 mg/l), which would have been limiting for standard growth conditions. The growth curve and enzyme assay indicate that the cells preferentially use up the inorganic phosphate. They in fact reach a stationary phase at a density of cells proportional to the inorganic phosphate concentration and much lower than the control. Up to this point no phosphatase activity could be detected. A second exponential growth followed, with the utilization of the β-glycerophosphate present in the medium as indicated by the simultaneous appearance of the alkaline phosphatase.

These results confirm the presence of an alkaline phosphatase in Chlamydomonas r. and that the inorganic phosphate regulates the appearance of this enzyme. In fact, there is enzymatic activity only when there is no inorganic phosphate in the culture medium, (Fig. 1c), or after the exhaustion of residual or limiting phosphate (Figs. 1b and 2c).

Furthermore, the carbon source does play an important role in the synthesis of the alkaline phosphatase. Comparison between Figs. 1b and 2b clearly indicates that the algae behave in two different ways. The culture grown in the presence of CO_2 shows a diauxy-type phenomenon and the enzyme appears only after 48 hours. The culture grown in the presence of acetate does not show this phenomenon and the alkaline phosphatase appears rapidly (12 h).

It is known that in organisms that can utilize either one of these carbon sources, growth in acetate implies some metabolic pathways that are different from those utilized when growth occurs in the presence of CO_2. This notion could account for the immediate response to induction by β-glycerophosphate and consequently the lack of a diauxy-type of curve in acetate-grown cells.

On the other hand, it is also possible to postulate that the

acetate functions as a co-inducer of a series of structural genes (aldolase II (6) and alkaline phosphatase), which could be switched on by its presence in the system.

Experiments are in progress to test these hypotheses.

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