THE RELATIONSHIP BETWEEN THE RATE OF PHOSPHATE ABSORPTION AND PROTEIN SYNTHESIS DURING PHOSPHATE STARVATION IN *CHLORELLA PYRENOIDOSA*

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1. Introduction

The elimination of essential anions such as phosphate or sulphate from the culture medium causes an increase in the rate of absorption of these ions in bacteria [1] yeast cells [2, 3] and higher plant cells [4–8]. This increase may be due to an increased synthesis of the absorption systems, to an activation of these systems, or to both mechanisms acting together. In order to find whether this phenomenon involves new protein synthesis, we attempted to interfere with protein synthesis by various means and investigated the effect of these treatments on the increase in the rate of phosphate absorption after phosphate starvation in *Chlorella pyrenoidosa*.

2. Methods and techniques

2.1. Cultures

The cells used were taken from cultures in the exponential growth phase. They were centrifuged and resuspended in a medium lacking phosphate [1]. The cells were routinely starved of phosphate in a media sparged with 2–3% CO_2 in air, in the light.

2.2. Phosphate uptake

Cells incubated in media with or without phosphate were centrifuged and resuspended in Tris-HCl (50 mM, pH 6,6). After 5 min agitation radioactive phosphate was injected; samples were then withdrawn at various times and filtered on millipore membranes. After drying, the radioactivity of the filtrate was measured either by a thin window counter or in a liquid scintillation counter.

2.3. Growth measurement

Cells were counted and their size determined, with a Coulter cell counter.

2.4. Radioactive solutions

^{32}P was supplied by the C.E.A. in the form $H_3{}^{32}PO_4$.

2.5. Presentation of results

Phosphate absorption rates are expressed as percent of the activity of non-phosphate-starved controls. The average values for the initial absorption rates (during the first minute) for a phosphate concentration of 2.5×10^{-5} M were $1.02 \times 10^{-11} \pm 0.24$ μmole/cell/min for non-starved and $6.2 \times 10^{-13} \pm 1.4$ μmole/cell/min for Chlorella starved of phosphate for 4 hr. When an external concentration of 10^{-3} M phosphate was used, the relative increase in phosphate absorption rate was the same.

3. Results

3.1. The role of carbon and sulphur metabolism in the increase in phosphate absorption rate during starvation

Selenate, which follows the same metabolic pathways as sulphate, interferes with the incorporation of sulphur amino acids into proteins. Experiments were carried out in which sulphate in the phosphate starvation medium was partially replaced by selenate (table 1). The results indicate that the presence of selenate reduced the increase of phosphate absorption rate.

After a period of adaptation, Chlorella cells are able to use methionine as a sulphur source. Using cells adapted in this way, phosphate starvation was carried out in media containing various analogues of methionine as sulphur sources. In the presence of selenomethionine, the increase in absorption rate was reduced, while ethionine had little effect (table 2).

When cells were starved in air levels of CO_2 or in darkness, the increase in the rate of phosphate absorption was very small (table 3). In the absence of high CO_2 levels and in darkness, less reduced carbon is available for protein synthesis: in addition, in darkness sulphate and nitrate reduction is slow. The lowered phosphate absorption rates observed after dark and low CO_2 treatments are thus explicable in terms of diminished protein synthesis.

Table 1
The role of sulphur metabolism in the increase in phosphate absorption rate during starvation.

Non starved cells	Starved cells	Starved cells in presence of selenate	
		1	2
100%	400%	180%	210%

Column 1: $SeO_4^{2-}/SO_4^{2-} = 1$, Column 2: $SeO_4^{2-}/SO_4^{2-} = 0.33$.

Table 2
The role of sulfur metabolism in the increase in phosphate absorption rate during starvation.

Non starved cells	Starved cells in presence of methionine	Starved cells in presence of ethionine	Starved cells in presence of seleno-methionine
100%	550%	410%	190%

Table 3
The role of carbon and darkness in the increase in phosphate absorption rate during starvation.

Non starved cells	Starved cells	Starved cells in air levels of CO_2	Starved cells in darkness
100%	460%	130%	140%

3.2. The effect of cycloheximide on the increase in absorption rate during starvation

Two batches of Chlorella cells were starved of phosphate for 4 hr in the presence or absence of cycloheximide (0.12 mg/ml); phosphate absorption rate was then determined using a range of phosphate con-

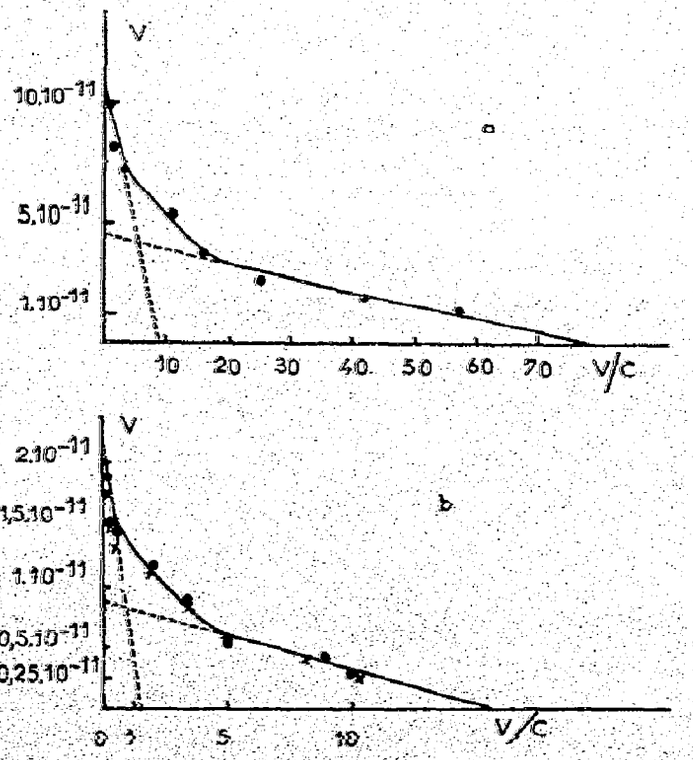

Fig. 1. The relationship between rate of phosphate absorption and phosphate concentration: V = f(V/C). V = rate of absorption in μmoles/cell/min; C = phosphate concentration (μM). a) Chlorella starved of phosphate for 4 hr; b) Chlorella starved of phosphate in the presence of cycloheximide; (×—×—×) non-starved Chlorella.

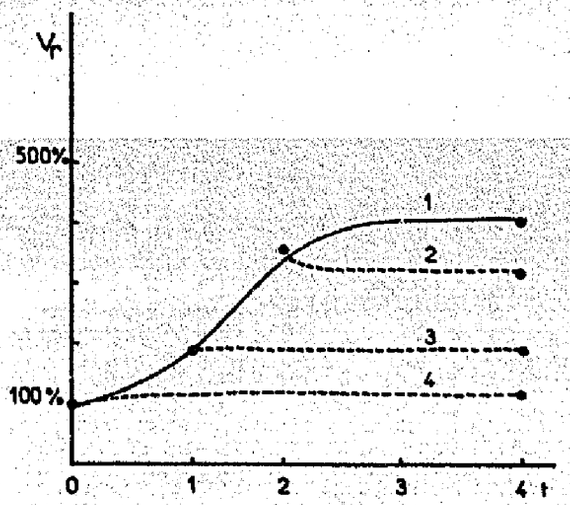

Fig. 2. The effect of cycloheximide on the rate of phosphate absorption. Cycloheximide was added at various times during phosphate starvation. Curve 1: time course of increase in phosphate absorption rate during phosphate starvation. Curve 2: cycloheximide addition after 2 hr of phosphate starvation. Curve 3: cycloheximide addition after 1 hr of phosphate starvation. Curve 4: Cycloheximide addition at the beginning of phosphate starvation. V_r: Relative phosphate absorption rate. Non-starved Chlorella taken as 100%; t: period of phosphate starvation (hr).

centrations. Fig. 1 presents the rates of absorption observed at different concentrations (at pH 6.6), using the relationship $V = f(V/C)$. Two slopes are apparent, which are interpreted as evidence for two types of absorption [9]. In the presence of cycloheximide, the increase in absorption rate did not occur. In this experiment, the cell number was 9×10^6 cells at the beginning and after phosphate starvation, in presence or absence of cycloheximide it was 1.04×10^7 and 1.20×10^7, respectively. When cycloheximide was added to the starvation medium at various times, the increase in absorption rate was immediately stopped although the absorption capacity already developed was not affected (fig. 2). This result strongly suggests that cycloheximide is acting at the level of synthesis of the absorption system.

4. Discussion

The increase in the rate of phosphate absorption after starvation for this ion can be attributed to a synthesis of absorption sites involving protein synthesis. The experiments with cycloheximide strongly support this argument, while those involving sulphur and carbon metabolism are consistent with it. Similar results have been obtained for hexose transport in animal cells [11].

In the case of Chlorella cells, a de-repression phenomenon like that seen in other algae [10] is apparently not involved as cells not starved of phosphate are able to absorb this ion. It seems possible that the absence of phosphate from the medium stimulates the synthesis of new absorption sites.

As we have previously shown that the addition of phosphate to starved Chlorella results in a reduction in the absorption rate, the presence of phosphate in the medium may repress the synthesis of the absorption sites until it reaches a base line. A complex control system regulating phosphate entry into the cell is implicated, in which protein synthesis is involved.

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