THE INFLUENCE OF LIGHT ON THE ACTIVITY OF NITRATE REDUCTASE IN SYNCHRONOUS CULTURES OF *CHLORELLA PYRENOIDOSA* *

M. HODLER, J.-J. MORGENTHALER†, W. EICHENBERGER and E.C. GROB‡
Department of organic Chemistry, University of Bern, Länggass-Str. 7, Bern, Switzerland

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1. Introduction

Previous experiments with *Spirodela oligorrhiza* plants grown on a medium containing nitrate and ammonium revealed that the uptake of nitrate is stimulated by exogenous glucose [1]. From these results the question arises as to whether glucose controls the uptake of nitrate.

In green plants the nitrate assimilation is usually controlled at the level of nitrate reduction, i.e. by control of the activity of nitrate reductase [2]. Since certain kinds of carbon metabolites have been found to influence the activity of this enzyme [3], glucose itself might also affect this step of the reaction.

In order to perform further experiments under defined physiological conditions synchronous cultures of *Chlorella pyrenoidosa* were used. Evidence is given by several papers that nitrate reductase of green plants is influenced primarily by light [4–11]. Before starting further experiments concerning the effect of glucose a precise study of the influence of light on nitrate reductase of *Chlorella* had to be done. As already demonstrated by Kanazawa et al. [12] pre-illuminated *Chlorella* cells incorporate $^{14}CO_2$ much more actively into amino acids than cells kept in the dark before incubation. From these facts the authors concluded that the utilization of nitrate was stimulated by light. Direct measurements of nitrate reductase activity, however, were not carried out. Therefore our contribution deals with the enzyme activity as influenced by light and dark.

2. Material and methods

Chlorella pyrenoidosa (strain 211-8k‡) was grown in an apparatus for continuous cultivation as described previously [13]. A light–dark ratio of 14:10 hr, respectively, was applied, resulting in a satisfactory synchronicity and avoiding the inconvenience of the 16:12 hr ratio [14]. The cells were grown in a modified Hutner's medium [15, 16] containing an equivalent total amount of nitrogen sources exclusively in the form of nitrate. The culture vessel was aerated by a mixture of air and CO_2 (100:3, v/v) at a rate of 10 ℓ/min.

The cells from 1200 ml of culture were routinely harvested by pelleting and washed twice with water. The cells were then resuspended in 4 ml of water and mixed with 10 g of glass beads (0.25–0.3 mm diameter) per g wet weight and broken in a Merkenschlager homogenizer [17] for 2 min. The whole system was kept cool with liquid CO_2. After decantation from the glass beads 10 ml of 5 mM Tris-HCl, pH 7.5 per g wet weight of cells were added and unbroken cells and cell debris were removed by centrifugation for 30 min at 23,000 *g*. Solid streptomycin sulfate was added to the turbid supernatant to a final conc. of 7 mM and after standing at room temp. for 10 min, the mixture was recentrifuged for 10 min at 23,000 *g* [18]. Enzyme assays

* Part III of a series on nitrogen metabolism in green plants. For part II see [1].

† Present address: Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, N.J., USA.

‡ Deceased May 1972.

‡ Culture collection, Institute of Plant Physiology, University of Göttingen, Germany.

were carried out with the clear yellow-colored supernatant, based on the procedure of Paneque et al. [19]. $FMNH_2$ was added as an electron donor, the regeneration of which was afforded by the presence of excess dithionite. The nitrite formed by the enzyme was converted to an azo-dye and determined photometrically. The standard deviation with 5 to 6 measurements was found to be ± 8%. All values were based on the protein content determined by TCA precipitation and Biuret-reagent [20]. Bovine serum albumin (Sigma) was used as a standard. The frequency of measurements was limited for technical reasons to one determination every 2 hr.

3. Results and discussion

The activity of nitrate reductase was determined in two subsequent series of experiments, i.e. at the beginning and at the end of the light period. As shown in fig. 1a, the enzyme activity of illuminated cells declines rapidly during the first hour of the dark period to a value of almost zero. Since in these synchronous cultures cell division coincides with the beginning of the dark period, we can not exclude any possible influence of the division process on the enzyme activity. If light alone were the inducing factor, the drop in activity should be inducible at any time by darkness, independently of cell division. In another experiment, therefore, darkness was started 4 hr earlier. In the latter case, an immediate decrease of enzyme activity at the onset of the dark period was again observed (fig. 1b), although cell division took place at the normal time [14]. The same effect on enzyme activity is also produced by darkness after an extended light period, although the culture in this case does not show a simultaneous cell division.

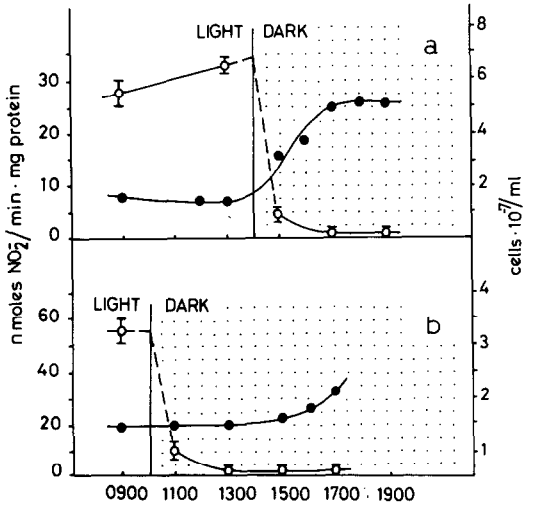

Fig. 1. Activity of nitrate reductase (○—○—○) and cell number (●—●—●) of synchronous cultures of *Chlorella pyrenoidosa* during light and dark. Light/dark change: a) regular time; b) 4 hr earlier.

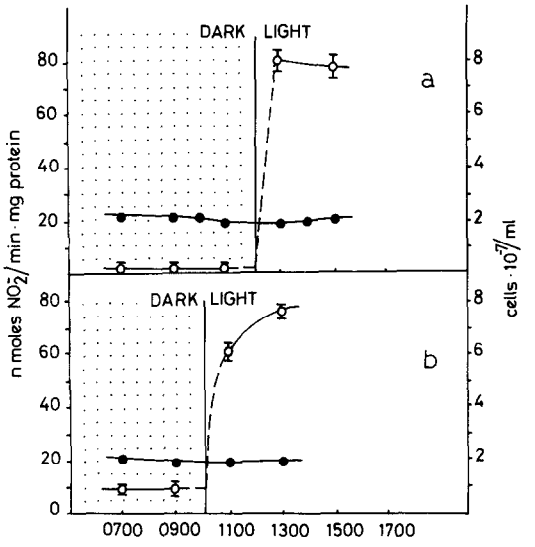

Fig. 2. Activity of nitrate reductase (○—○—○) and cell number (●—●—●) of synchronous cultures of *Chlorella pyrenoidosa* during light and dark. Dark/light change: a) regular time; b) 2 hr earlier.

At the beginning of the light period, as demonstrated by fig. 2a, nitrate reductase activity increases rapidly. The same increase is also observed if illumination is started 2 hr earlier (fig. 2b).

Immediate changes of enzyme activity on illumination or darkness show clearly that the activity of nitrate reductase is strictly light-dependent in *Chlorella*. This implies that in these cells nitrate reduction is only possible on illumination.

A similar light-dependence of nitrate reductase has been found in several higher plants [4–7, 9, 11], producing daily fluctuations of the enzyme activity [8]. On the other hand, corn seedlings grown under aerobic conditions produce an active nitrate reductase also in the dark [10]. In rice seedlings the activity of this en-

zyme is light-dependent until the age of 7 days, but light-dependent again in older plants [21]. From these and other studies [2, 21, 22], the exact mechanism of the influence of light on nitrate reductase is still far from being clear. Among the mechanisms that have been proposed the photochemical formation of reducing power which then would act as an electron donor for the enzyme has been invoked. However, in our system, $FMNH_2$ acting as a primary electron donor [23], is present in excess in the assay system and therefore not limiting. Moreover, our results clearly demonstrate a relatively quick response of enzyme activity to light and dark. Within 1 hr of illumination or darkness 80 to 100% of enzyme activity is attained or lost. In contrast, in the experiments of Kanazawa et al. [12] *Chlorella* did not show any response after 45 min of illumination, while the response of nitrate reductase of higher plants seems to be even slower [6, 7, 11]. The short lag period of our system might suggest a mechanism which is different from that in higher plants. It also makes control by light at the level of protein synthesis quite unlikely. Since more specific information is needed for the exact time course of enzyme activity, measurements at shorter time intervals are being carried out at present.

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